CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Solanum lycopersicum L.

TOMATO

UPOV Code: SOLAN_LYC

Adopted on 17/06/2026

Entry into force on 01/06/2026

CPVO-TP/044/5-Rev based on the version 6 of the CPVO-TP template

** These names were correct at the time of the introduction of the protocol but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]*
*** The names of pests and diseases were correct at the time of the introduction of these Test Guidelines but may be revised or updated. Readers are advised to consult the 'Recommended Codes for Pest Organisms in Vegetable Crops', which can be found on the ISF Website (https://worldseed.org/), for the latest information.*

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The technical examination shall be carried out in accordance with the provisions of the Designation Agreement and of the Entrustment requirements (Quality Audit Service | CPVO) applicable at the time the DUS technical examination takes place.

1. SUBJECT OF THE PROTOCOL

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of ***Solanum lycopersicum*** **L.,** ***Solanum lycopersicum*** **L. x** ***Solanum cheesmaniae*** **(L. Ridley) Fosber and** ***Solanum lycopersicum*** **L. x** ***Solanum pimpinellifolium*** **L.** (including rootstocks of these species).

For tomato rootstock varieties belonging to other species CPVO/TP-294 applies.

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS examination (UPOV Document TG/1/3 (link), its associated TGP documents (link) and the relevant UPOV Test Guideline TG/44/12 rev. dated 21/10/2025 (https://www.upov.int/documents/d/upov/tg-documents-en-tg044.pdf) for the conduct of tests for Distinctness, Uniformity and Stability (DUS).

1.2 Entry into Force

The present protocol enters into force on **01.06.2026**.

Technical examinations of candidate varieties are carried out according to the TP in force when the DUS examination starts. The starting date of a DUS examination is considered to be the final date for submitting of plant material for the first growing cycle.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on https://public.plantvarieties.eu/publication in the special issue S2/S3 of the Official Gazette of the Office. General requirements on submission of plant material are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO requests the plant material to the applicant in accordance with paragraph 2.1.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

3.1.1 The minimum duration of tests should normally be two independent growing cycles.

Two growing cycles do not necessarily mean that each characteristic is observed in the same way twice, especially when it has been demonstrated that a second observation will not provide more certainty or a different result regarding distinctness, uniformity and stability, or the description of the characteristic. Further specification is provided in the respective explanations for individual characteristics in Chapter 8.

3.1.2 The two independent growing cycles should be in the form of two separate plantings.

3.1.3 The testing of a variety may be concluded when the Examination Office can determine with certainty the outcome of the test.

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness" http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf.

3.3 Conditions for Conducting the Examination

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 20 spaced plants, which should be divided between at least 2 replicates.

3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Additional characteristics

An applicant may claim, or the examination office may propose the use of an additional characteristic to establish distinctness. The observation of the additional characteristic will be undertaken only in case distinctness is unlikely to be shown using the characteristics listed in the protocol.

Under the conditions that the claim is supported by technical evidence and that a test procedure can be devised, the President of the CPVO may approve the use of such characteristic in accordance with Article 23 of Implementing Rules N° 874/2009.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

4.1 Distinctness

4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document), TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability". and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness.

4.1.2 Consistent differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts taken from each of 10 plants and any other observations made on all plants in the test, disregarding any off-type plants.

For testing the resistance to certain pathogens, unless otherwise indicated, the test should be performed on at least 20 plants.

4.1.5 Method of observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the third column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "Observation of characteristics"):

MG: single measurement of a group of plants or parts of plants
MS: measurement of a number of individual plants or parts of plants
VG: visual assessment by a single observation of a group of plants or parts of plants
VS: visual assessment by observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)
"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. colour charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)
For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, "G" provides a single record per variety, and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness.

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

4.2 Uniformity

4.2.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity.

4.2.2 This Technical Protocol has been developed for the examination of seed-propagated and vegetatively propagated varieties. For varieties with other types of propagation the recommendations in the UPOV-General Introduction to DUS and document TGP/13 "Guidance for new types and species", Section 4.5 "Testing Uniformity" should be followed.

4.2.3 For the assessment of uniformity of self-pollinated varieties, single cross hybrids and vegetatively propagated varieties, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 20 plants, 1 off-type is allowed.

4.3 Stability

4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pd)

In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of varieties, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. GROUPING OF VARIETIES AND ORGANISATION OF THE GROWING TRIAL

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organise the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

a) Plant: growth type (characteristic 2)

b) Leaf: type (characteristic 10)

c) Pedicel: abscission layer (characteristic 18)

d) Immature fruit: green shoulder (characteristic 20)

e) Immature fruit: green stripes (characteristic 24)

f) Immature fruit: anthocyanin coloration (characteristic 25)

g) Fruit: size (characteristic 26)

h) Fruit: shape in longitudinal section (characteristic 28)

i) Fruit: number of locules (characteristic 36)

j) Fruit: gel in locules (characteristic 37)

k) Fruit: colour (characteristic 38)

l) Resistance to *Meloidogyne incognita* (Mi) (characteristic 45)

m) Resistance to *Verticilium* sp. (Va and Vd) – Race 0 (characteristic 46)

n) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 0EU/1US (Fol: 0EU/1US) (characteristic 47)

o) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 1EU/2US (Fol: 1EU/2US) (characteristic 48)

p) Resistance to Tomato mosaic virus – Strain 0 (ToMV: 0) (characteristic 60)

q) Resistance to Tomato spotted wilt virus – Pathotype 0 (TSWV: 0) (characteristic 69)

5.4 If characteristics other than those mentioned in the list of grouping characteristics and/or from the table of characteristics and/or from the Technical Questionnaire – sections 5 and 7 are used for the selection of varieties to be included into the growing trial, the Examination Office shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

5.5 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

The characteristics to be used in DUS examinations and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed.

Notwithstanding the above, the observation of characteristics labelled with the symbol '**nc**' in the table of characteristics is not compulsory.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

6.2. States of expression and corresponding notes

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description. All relevant states of expression are presented in the characteristic.

Further explanation of the presentation of states of expression and notes is provided in UPOV document TGP/7 "Development of Test Guidelines".

6.3 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.4 Legend

For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2
nc	Not compulsory characteristic	-see Chapter 6.1

For column 'UPOV N°':

The numbering of the characteristics is provided as a reference to the UPOV guideline.

(*)	UPOV Asterisked characteristic	-Characteristics that are important for the international harmonization of variety descriptions.

For column 'Stage, method':

MG, MS, VG, VS		-see Chapter 4.1.5
(a)-(c)	Explanations covering several Characteristics	-see Chapter 8.1

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1. (+)	**1.**	**VS**	**<u>Seed-propagated varieties only</u>: Seedling: anthocyanin coloration of hypocotyl**		
QN			absent	Colt, VTM215	1
			partially present		2
			totally present	Daniela, Marmande VR	3
2. (+)	**2. (*)**	**VG**	**Plant: growth type**		
QL			determinate	Rio Grande, Siluet	1
G			indeterminate	Daniela, Florenteen, Marmande VR, Saint-Pierre	2
3. (+)	**3. (*)**	**MS/VG**	**<u>Only varieties with plant growth type determinate</u>: Plant: number of inflorescences on main stem**		
QN			very few	Cherry Falls	1
			very few to few	Monty	2
			few	Simplex	3
			few to medium		4
			medium	Miceno	5
			medium to many		6
			many	Malkonet	7
			many to very many	Grownet	8
			very many		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
4. (+)	**4.**	**VG**	**Stem: anthocyanin coloration**		
QN			absent or very weak	Rebelski	1
			very weak to weak		2
			weak	Montfavet 63-5	3
			weak to medium		4
			medium	Miniprio, Philovita	5
			medium to strong		6
			strong	Grinta	7
			strong to very strong		8
			very strong	Villax	9
5. (+)	**5.**	**MS/VG**	**Only varieties with plant growth type indeterminate: Stem: length of internode**		
QN			very short		1
			very short to short		2
			short	Primioso	3
			short to medium		4
			medium	Campari, Montfavet 63-5	5
			medium to long		6
			long	Rebelski, Tomawak	7
			long to very long		8
			very long		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6. (+)	**6. (*)**	**MS/VG**	**<u>Only varieties with plant growth type indeterminate:</u> Plant: height**		
QN			very short	Gardener's Delight, Maresme, Zadenna	1
			very short to short		2
			short	Delfine, Despina	3
			short to medium		4
			medium	Brooklyn, Campari	5
			medium to tall		6
			tall	Climberley, Pitenza	7
			tall to very tall		8
			very tall	Goldwin, Romindo	9
7. (+)	**7. (*)**	**VG**	**Leaf: attitude**		
QN		**(a)**	erect		1
			erect to semi-erect		2
			semi-erect	Zadenna	3
			semi-erect to horizontal		4
			horizontal	Brioso, Geronimo	5
			horizontal to semi-drooping		6
			semi-drooping	Leonce, Montfavet 63-5, Upper	7
			semi-drooping to drooping		8
			drooping	Caboverde	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
8.	**8.**	**MS/VG**	**Leaf: length**		
QN		**(a)**	very short		1
			very short to short		2
			short	Red Robin	3
			short to medium		4
			medium	Mezcal, Rio Grande	5
			medium to long		6
			long	Geronimo, Montfavet 63-5	7
			long to very long		8
			very long		9
9.	**9.**	**MS/VG**	**Leaf: width**		
QN		**(a)**	very narrow		1
			very narrow to narrow		2
			narrow	Red Robin	3
			narrow to medium		4
			medium	Rio Grande	5
			medium to broad		6
			broad	Brioso, Saint-Pierre	7
			broad to very broad		8
			very broad		9
10. (+)	**10. (*)**	**VG**	**Leaf: type**		
QL		**(a)**	pinnate	Matina	1
G			bipinnate	Daniela, Saint-Pierre	2

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
11. (+)	**11.**	**VG**	**Leaf: size of leaflets**		
QN		**(a)**	very small	Micro Tom	1
			very small to small		2
			small	Tiny Tim	3
			small to medium		4
			medium	Geronimo, Marmande VR	5
			medium to large		6
			large	Daniela	7
			large to very large		8
			very large		9
12.	**12. (*)**	**VG**	**Leaf: intensity of green colour**		
QN		**(a)**	very light		1
			very light to light		2
			light	Rossol	3
			light to medium		4
			medium	Rebelski	5
			medium to dark		6
			dark	Daniela, Red Robin	7
			dark to very dark		8
			very dark		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
13. (+)	**13.**	**VG**	**Leaf: glossiness**		
QN		**(a)**	very weak	Speedax	1
			very weak to weak		2
			weak	Daniela, Losna	3
			weak to medium		4
			medium	Marmande VR	5
			medium to strong		6
			strong	Albis, Dulcemiel, Lutecia	7
			strong to very strong	Wasino	8
			very strong		9
14. (+)	**14.**	**VG**	**Leaf: blistering**		
QN		**(a)**	very weak		1
			very weak to weak		2
			weak	Daniela	3
			weak to medium		4
			medium	Marmande VR, Octavio, Syrio	5
			medium to strong		6
			strong	Albis, Delfine, Paronset, Red Robin	7
			strong to very strong		8
			very strong		9
15. (+)	**15.**	**VG**	**Leaf: attitude of petiolule of leaflets in relation to petiole**		
QN		**(a)**	erect	Volantis	1
			erect to semi-erect		2
			semi-erect	Geronimo, Marmande VR	3
			semi-erect to horizontal		4
			horizontal	Delisher	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
16. (+)	**16.**	**MS/VG**	**Inflorescence: type**		
PQ			mainly uniparous	Geronimo, Red Robin	1
			equally uniparous and multiparous	Harzfeuer	2
			mainly multiparous	Karelya	3
			multiflora	Mini Star, Sweedor	4
17.	**17. (*)**	**VG**	**Flower: colour**		
QL			yellow	Marmande VR, Santorange	1
			orange	Mountain Vineyard, Orama	2
18. (+)	**18. (*)**	**VG**	**Pedicel: abscission layer**		
QL			absent	Merlice, Rio Grande	1
G			present	Daniela, Grownet, Montfavet 63-5	9
19. (+)	**19. (*)**	**MS/VG**	**<u>Only for varieties with pedicel abscission layer present</u>: Pedicel: length**		
QN			very short		1
			very short to short		2
			short	Cerise, Ferline	3
			short to medium		4
			medium	Caboverde, Grownet	5
			medium to long		6
			long	Sir Elyan	7
			long to very long		8
			very long		9
20. (+)	**20. (*)**	**VG**	**Immature fruit: green shoulder**		
QL		**(b)**	absent	Geronimo	1
G			present	Daniela, Montfavet 63-5	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
21. (+)	**21.**	**VG**	**Immature fruit: extent of green shoulder**		
QN		**(b)**	very small	Daniela	1
			very small to small		2
			small	Shiren, Siluet	3
			small to medium		4
			medium	Marmalindo, Montfavet 63-5, Red Robin	5
			medium to large		6
			large	Cobra, Dulcemiel	7
			large to very large		8
			very large		9
22. (+)	**22.**	**VG**	**Immature fruit: intensity of green colour of shoulder**		
QN		**(b)**	very light		1
			very light to light		2
			light	Daniela, Soltyno	3
			light to medium		4
			medium	Montfavet 63-5, Santonio, Sunita	5
			medium to dark		6
			dark	Brito, Nugget	7
			dark to very dark		8
			very dark		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
23. (+)	**23. (*)**	**VG**	**Immature fruit: intensity of green colour excluding shoulder**		
QN		**(b)**	very light	Claree	1
			very light to light		2
			light	Daniela, Durinta, Trust	3
			light to medium		4
			medium	Sunita, Tropical	5
			medium to dark		6
			dark	Centella, Chocomate, Uragano	7
			dark to very dark		8
			very dark	Momi, Verdi	9
24.	**24 (*)**	**VG**	**Immature fruit: green stripes**		
QL		**(b)**	absent	Daniela, Guanche, Jasminia	1
G			present	Green Zebra, Tigerella	9
25.	**25 (*)**	**VG**	**Immature fruit: anthocyanin coloration**		
QL		**(b)**	absent	Durinta	1
G			present	HN5003	9
26.	**26. (*)**	**MS/VG**	**Fruit: size**		
QN		**(c)**	very small	Cerise, Sweet 100	1
			very small to small	Dolcetini, Genio	2
			small	Brioso, Tankini	3
			small to medium	Larimar, Progress	4
			medium	Mezcal, Oceano	5
			medium to large	Luminance, Rio Grande	6
			large	Carmello, Floradade	7
			large to very large	Florenteen, Grownet	8
G			very large	Cupidissimo, Marsilia	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
27.	**27. (*)**	**MS/VG**	**Fruit: ratio length/diameter**		
QN		**(c)**	very low	Margold, Marmande VR	1
			very low to low	Lutecia, Shourouq	2
			low	Cupidissimo, Motto	3
			low to medium	Kaponet, Laureen, Merlice	4
			medium	Chocostar, Mezcal, Red Robin	5
			medium to high	Dulcini, Ibix	6
			high	Oceano, Oribustar, Rio Grande	7
			high to very high	Ibrax, Sir Elyan	8
			very high	Bellandine, Capriccio, Elko	9
28. (+)	**28. (*)**	**VG**	**Fruit: shape in longitudinal section**		
PQ		**(c)**	flattened	Margold, Marmande VR	1
			oblate	Cartesio, Gloriette, Merlice, Montfavet 63-5	2
			circular	Cerise, Soussia	3
			oblong	Landolino, Red Sky	4
			cylindric	Hypeel 244, Sir Elyan	5
			elliptic	Obock	6
			cordate	Cuor di Bue, Cupidissimo, Laureen, Valenciano	7
			ovate	Dualrow, Soto	8
			obovate	Duquesa, Estelle, Mezcal	9
			pyriform	Oceano, Olivenza, Operino	10
G			obcordate	Cuore del Ponente, Ingrid	11

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
29. (+)	**29. (*)**	**VG**	**Fruit: ribbing**		
QN		**(c)**	absent or very weak	Cerise, Conchita	1
			very weak to weak		2
			weak	Baikonur, Guanche	3
			weak to medium		4
			medium	Montfavet 63-5, Shourouq	5
			medium to strong		6
			strong	Marmalindo, Marmande VR, Marsilia	7
			strong to very strong		8
			very strong	Ingrid, Marsalato	9
30. (+)	**30.**	**VG**	**Fruit: depression at pedicel end**		
QN		**(c)**	absent or very weak	Mirante, Sweet Baby	1
			very weak to weak		2
			weak	Bodega, Lebron, Melody	3
			weak to medium		4
			medium	Fandango, Hibisco, Jasminia, Saint-Pierre	5
			medium to strong		6
			strong	Igido, Losna, Marmande VR	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
31. (+)	**31.**	**MS/VG**	**Fruit: size of pedicel scar**		
QN		**(c)**	very small	Cerise, Sweet Baby	1
			very small to small		2
			small	Cherrubino, Tukami	3
			small to medium		4
			medium	Bodega, Hibisco, Montfavet 63-5	5
			medium to large		6
			large	Fandango, Gloriette, Jasminia	7
			large to very large		8
			very large	Baikonur, Ensemble, Marmande VR	9
32.	**32.**	**MS/VG**	**Fruit: size of blossom scar**		
QN		**(c)**	very small	Cerise, Conchita, Mirante	1
			very small to small		2
			small	Ensemble, Lilos, Montfavet 63-5	3
			small to medium		4
			medium	Pink Bisou	5
			medium to large		6
			large	Esmira, Marinda, Marmande VR, Saint-Pierre	7
			large to very large		8
			very large	Marsalato, Marsilia	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
33. (+)	**33.**	**VG**	**Fruit: shape at blossom end**		
QN		**(c)**	indented	Marmande VR	1
			indented to flat	Framboo, Linnea	2
			flat	Montfavet 63-5, Realeza, Viniccio	3
			flat to pointed	Batistuta	4
			pointed	Roma VF, Talentum	5
34. (+)	**34.**	**MS/VG**	**Fruit: diameter of core in cross section in relation to total diameter**		
QN		**(c)**	very small	Cerise	1
			very small to small		2
			small	Dolce Vita, Takumi	3
			small to medium		4
			medium	Losna, Montfavet 63-5, Tastery	5
			medium to large		6
			large	Commodo, Paradigma	7
			large to very large		8
			very large	Baikonur, Marmande VR, Valenciano	9
35. (+)	**35.**	**VG**	**Fruit: thickness of pericarp**		
QN		**(c)**	very thin	Cerise	1
			very thin to thin		2
			thin	Astuto, Conchita, Marmande VR	3
			thin to medium		4
			medium	Jayran, Montfavet 63-5, Refosco	5
			medium to thick		6
			thick	Losna, Reconquista	7
			thick to very thick		8
			very thick	Delibes, Floyd, Myriade, Orinade	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
36. (+)	**36. (*)**	**MS/VG**	**Fruit: number of locules**		
QN		**(c)**	only two	Creativo, San Marzano 2, Tropical	1
			two and three	Bomfado, Orinade	2
			three and four	Durinta, Montfavet 63-5	3
			four, five or six	Rovente, Tosmar, Tradiro	4
G			more than six	Bronson, Chocostar, Marmande VR	5
37. (+)	**37. (*)**	**VG**	**Fruit: gel in locules**		
QL		**(c)**	absent	Allflesh 1120, Nun 03560	1
G			present	Daniela, Rio Grande	9
38. (+)	**38. (*)**	**VG**	**Fruit: colour**		
PQ		**(c)**	yellowish white	Cream Sausage	1
			yellow	Babylor, Mimosa	2
			orange	Operino, Oranjestar	3
			pink	Framboo, Pink Wand, Tomimaru Muchoo	4
			red	Daniela, Ferline, Montfavet 63-5, Saint-Pierre, Umaca	5
			brown	Chocostar, Marbruni	6
G			green	Green Grape, Green Zebra	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
39. (+)	**39.**	**VG**	**Fruit: colour of flesh**		
PQ		**(c)**	yellowish white	Cream Sausage	1
			yellow	Babylor, Mimosa	2
			orange	Operino, Oranjestar	3
			pink	Framboo, Pink Wand	4
			red	Daniela, Ferline, Montfavet 63-5, Saint-Pierre, Tomimaru Muchoo, Umaca	5
			brown	Chocostar, Marbruni	6
			green	Green Grape, Green Zebra	7
40. (+)	**40.**	**VG**	**Fruit: glossiness of skin**		
QN		**(c)**	weak	Focale, Josefina, Sylvana	1
			medium	Ventero	2
			strong	Daltoma, Mecano	3
41. (+)	**41. (*)**	**VG**	**Fruit: colour of epidermis**		
QL		**(c)**	colourless	Black Opal, Fruits, House Momotaro, Marvori	1
			yellow	Brown Berry, Daniela	2
42. (+)	**42. (*)**	**VG**	**Fruit: firmness**		
QN		**(c)**	very soft	Marmande VR	1
			very soft to soft		2
			soft	Marinda, Marsalato	3
			soft to medium		4
			medium	Rosannita, Sunita	5
			medium to firm		6
			firm	Losna, Octavio, Tradiro	7
			firm to very firm		8
			very firm	Brito, Daniela, Larimar, Lolek	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
43. (+)	**43.**	**MG/MS**	**Time of flowering**		
QN			very early	Pyremello, Trambellino	1
			very early to early	Creativo, Tropical	2
			early	Delizia, Lemonade, Zorayda	3
			early to medium	Cindel, Goldwin, Organza	4
			medium	Delisher, Losna, Montfavet 63-5, Sonico	5
			medium to late	Orama, Soltyno	6
			late	Octydia, Raymos, Saint-Pierre Sylvana	7
			late to very late	Nissos, Paronset	8
			very late	Atago, Brito, Wafira	9
44. (+)	**44. (*)**	**MG**	**Time of maturity**		
QN			very early	Goldwin, Pyremello, Sweet Baby, Trambellino	1
			very early to early	Delisher	2
			early	Lemonade, Shiren, Zorayda	3
			early to medium		4
			medium	Delizia, Losna, Sonico	5
			medium to late		6
			late	Mariana, Saneh	7
			late to very late		8
			very late	Atago, Brito, Daniela, Raymos, Wafira	9
45. (+)	**45.**	**MS/VG /VS**	**Resistance to *Meloidogyne incognita* (Mi)**		
QN			absent or low	Casaque Rouge	1
			medium	Campeon, Tyonic	2
G			high	Anahu, Anahu x Casaque Rouge	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
46. (+)	**46.**	**VS/VG**	**Resistance to *Verticillium* sp. (Va and Vd) - Race 0**		
QL			absent	Marmande verte, Moneymaker	1
G			present	Marmande VR, Monalbo	9
47. (+)	**47.**	**VS/VG**	**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* - Race 0EU/1US (Fol: 0EU/1US)**		
QL			absent	Marmande verte, Moneymaker	1
G			present	Anabel, Marporum, Marsol	9
48 (+)	**48**	**MS/VS /VG**	**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* - Race 1EU/2US (Fol: 1EU/2US)**		
QL			absent	Marmande verte, Moneymaker	1
G			present	Motelle	9
49. (+) nc	**49.**	**MS/VS /VG**	**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* - Race 2EU/3US (Fol: 2EU/3US)**		
QL			absent	Marmande verte, Motelle	1
			present	Alliance, Ivanhoé	9
50. (+) nc	**50.**	**VS/VG**	**Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (For)**		
QL			absent	Moneymaker, Motelle	1
			present	Momor	9
51. (+) nc	**51.**	**MS/VS /VG**	**Resistance to *Passalora fulva* (Pf) - Race 0**		
QL			absent	Monalbo, Moneymaker	1
			present	Antique, Pink Treat, Retinto, Sprigel, Triatlon	9
52. (+) nc	**52.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race A**		
QL			absent	Monalbo, Moneymaker, Retinto	1
			present	Antique, Pink Treat, Sprigel, Triatlon	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
53. (+) nc	**53.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race B**		
QL			absent	Monalbo, Moneymaker, Pink Treat	1
			present	Antique, Retinto, Sprigel, Triatlon	9
54. (+) nc	**54.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race C**		
QL			absent	Monalbo, Moneymaker, Pink Treat, Retinto	1
			present	Antique, Sprigel, Triatlon	9
55. (+) nc	**55.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race D**		
QL			absent	Monalbo, Moneymaker, Triatlon	1
			present	Antique, Pink Treat, Retinto, Sprigel	9
56. (+) nc	**56.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race E**		
QL			absent	Monalbo, Moneymaker	1
			present	Antique, Sprigel	9
57. (+) nc	**57.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race F**		
QL			absent	Monalbo, Moneymaker	1
			present	Chelino, Completo	9
58. (+) nc	**58.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race H**		
QL			absent	Sprigel	1
			present	Chelino, Completo	9
59. (+) nc	**59.**	**VS/VG**	**Resistance to *Passalora fulva* (Pf) - Race J**		
QL			absent	Chelino, Completo	1
			present	Mogami	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
60. (+)	**60.**	**MS/VS /VG**	**Resistance to Tomato mosaic virus - Strain 0 (ToMV: 0)**		
QL			absent	Monalbo, Moneymaker	1
G			present	Mobaci, Mocimor, Momor, Moperou	9
61. (+) nc	**61.**	**MS/VS /VG**	**Resistance to Tomato mosaic virus - Strain 1 (ToMV: 1)**		
QL			absent	Mobaci, Monalbo, Moneymaker	1
			present	Mocimor, Momor, Moperou	9
62. (+) nc	**62.**	**MS/VS /VG**	**Resistance to Tomato mosaic virus - Strain 2 (ToMV: 2)**		
QL			absent	Monalbo, Moneymaker, Moperou	1
			present	Mobaci, Mocimor, Momor	9
63. (+) nc	**63.**	**VS/VG**	**Resistance to *Phytophthora infestans* (Pi)**		
QL			absent	Moneymaker, Saint Pierre	1
			present	Phantasia, Sixtina	9
64. (+) nc	**64.**	**VS/VG**	**Resistance to *Pseudopyrenochaeta lycopersici* (ex *Pyrenochaeta lycopersici)* (Pl)**		
QL			absent	Marmande verte	1
			present	Garance	9
65. (+) nc	**65.**	**VS/VG**	**Resistance to *Stemphylium* spp. (Ss)**		
QL			absent	Monalbo	1
			present	Motelle	9
66. (+) nc	**66.**	**VS/VG**	**Resistance to *Pseudomonas syringae* pv. *tomato* (Pst)**		
QL			absent	Monalbo, Moneymaker	1
			present	Fuzzer	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
67. (+) nc	**67.**	**VS/VG**	**Resistance to *Ralstonia solanacearum* – Race 1 (Rs: 1)**		
QL			absent	Floradel	1
			present	Caraïbo	9
68. (+) nc	**68.**	**VS/VG**	**Resistance to Tomato yellow leaf curl virus (TYLCV)**		
QL			absent	Marmande, Moneymaker	1
			present	Delyca, Montenegro	9
69. (+)	**69.**	**MS/VS /VG**	**Resistance to Tomato spotted wilt virus - Pathotype 0 (TSWV: 0)**		
QL			absent	Moneymaker, Montfavet 63-5, Mountain Magic	1
G			present	Bodar, Mospomor	9
70. (+) nc	**70.**	**VS/VG**	**Resistance to *Leveillula taurica* (Lt)**		
QL			absent	Montfavet 63-5	1
			present	Radiance	9
71. (+) nc	**71.**	**VS/VG**	**Resistance to *Pseudoidium neolycopersici* (ex *Oidium neolycopersici*) (Pn) (ex On)**		
QL			absent	Montfavet 63-5	1
			present	Romiro	9
72. (+) nc	**72.**	**VS/VG**	**Resistance to Tomato torrado virus (ToTV)**		
QL			absent	Daniela	1
			present	Matias	9

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

Characteristics containing the following key in the third column of the Table of Characteristics should be examined as indicated below:

(a) In the case of indeterminate varieties, observations should be made after a fruit set on at least five trusses and before ripening of the second truss. In the case of determinate varieties, all observations should be made after a fruit set on the second truss. Observations should be made in the middle third of the plant, before leaves senesce.

(b) Observations should be made on fully developed immature fruits.

(c) Observations should be made on mature fruits from the second or higher truss, avoiding first and last mature fruits on truss.

8.2 Explanations for individual characteristics

Ad. 1: Seed-propagated varieties only: Seedling: anthocyanin coloration of hypocotyl

Observations should be made on the hypocotyl, before development of the first leaves.

In heterozygous genotypes, anthocyanin coloration of hypocotyl may segregate. If the segregation occurs in the predicted manner, the variety should be classified as partly present. Presence of anthocyanin is caused by one dominant allele.

Ad. 2: Plant: growth type

Determinate (1):
The number of trusses is limited and differs between varieties. The number of leaves or internodes between inflorescences is irregular within a plant and varies from one to three. The stem ends with an inflorescence, and no lateral shoots are produced.

Indeterminate (2):
As a rule, the number of leaves or internodes between inflorescences is three. After every group of three leaves, three buds are developed: the terminal bud is transformed into an inflorescence, and stem elongation continues from one of the lateral buds. There is continuous growing with repetition of this growth pattern.

Sometimes only two leaves or internodes might be observed between inflorescences in some parts of plants (e.g. varieties originating from 'Daniela').

Ad. 3: Only varieties with plant growth type determinate: Plant: number of inflorescences on main stem

Observations can only be made if side shoots have been removed in the growing trial.

Ad. 4. Stem: anthocyanin coloration

Indeterminate growth type varieties: observations should be made around flowering of the third or fourth truss, on the upper third of the plant.

Determinate growth type varieties: observation should be made before the main stem stops growing, showing then truss/leaf division, on the upper third of the plant.

Ad. 5: Only varieties with growth type indeterminate: Stem: length of internode

Observation should be made at one time for the whole trial, e.g. after a fruit set on approximately 5 nodes.

The total length of the stem should be observed/measured between the first and fourth truss. When this observation/measure is divided by the number of internodes in between, an indication of the length of the internode is given.

Ad. 6: Only varieties with growth type indeterminate: Plant: height

Observations should be made at one time for the whole trial: 60 days after planting, or after a fruit set on approximately 5 nodes, or when the first variety in the trial has reached the wire in the green house or the top of the stake.

Ad. 7: Leaf: attitude

The attitude of the middle third part of the leaves with respect to the main stem should be observed. The line in picture indicates the angle between the stem and leaf (middle third of leaf).

3
semi-erect

5
horizontal

7
semi-drooping

9
drooping

Ad. 10: Leaf: type

Pinnate leaf: primary leaflets do not bear secondary leaflets.
Bipinnate leaf: primary leaflets are pinnate and bear secondary leaflets.

1
pinnate

2
bipinnate

Ad. 11: Leaf: size of leaflets

Observations should be made in the middle of the leaf.

Ad. 13: Leaf: glossiness

Observations should be made on leaves from the middle of the plant.

Ad. 14: Leaf: blistering

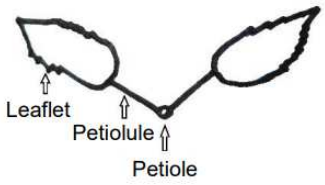
Observations should be made on leaves from the middle of the plant.
Caution is advised regarding the confusion between blistering and creasing.
Blistering is the difference in height of the surface of the leaf between the veins.
Creasing is independent from the veins.

blistering

creasing

Ad. 15: Leaf: attitude of petiolule of leaflet in relation to petiole

1
erect



3
semi-erect

5
horizontal

Ad. 16: Inflorescence: type

Observations should be made after fruit setting on the second and third trusses. If there is no predominant type, the variety should be described with state 2.

uniparous

multiparous (biparous)

multiparous (triparous)

multiflora

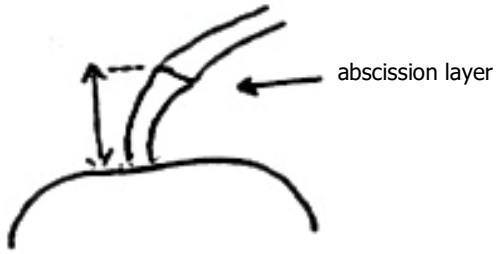
Ad. 18: Pedicel: abscission layer

Varieties without an abscission layer have only a collar on the pedicel.

1
absent

9
present

Ad. 19: Only varieties with pedicel abscission layer present: Pedicel: length



Observations should be made from the base until the abscission layer on harvested fruits.

Varieties which have only a collar instead of an abscission layer are heterozygous for the gene which controls the presence of the joint. These varieties are considered jointless and the abscission layer is considered absent.

Ad. 20: Immature fruit: green shoulder

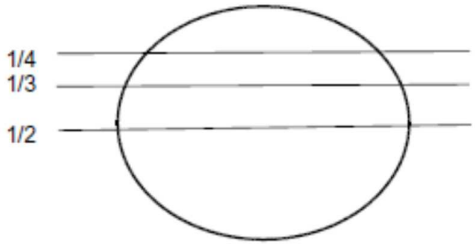
Due to potential environmental effects, example varieties should be included in the trial.

1
absent

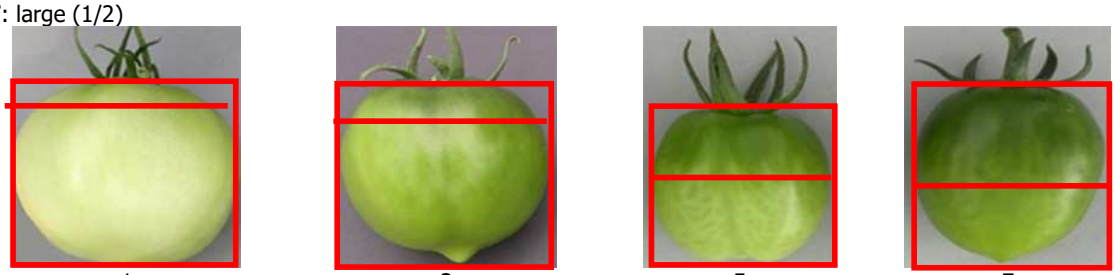
9
present

Ad. 21: Immature fruit: extent of green shoulder

Due to potential environmental effects, example varieties should be included in the trial.



3: small (1/4)
5: medium (1/3)
7: large (1/2)



1
very small

3
small

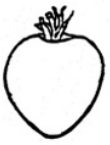
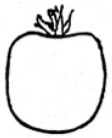
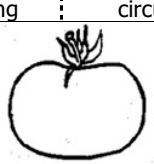
5
medium

7
large

Ad. 22: Immature fruit: intensity of green colour of shoulder
Ad. 23: Immature fruit: intensity of green colour excluding shoulder

Intensity of green colour of shoulder and intensity of green colour excluding shoulder have to be observed on the same scale. This means that the note for intensity of green colour of shoulder should be higher than the note for intensity of green colour excluding shoulder, or in exceptional cases the same if the difference in intensity is very small. Due to potential environmental effects, example varieties should be included in the trial.

Ad. 28: Fruit: shape in longitudinal section

	← broadest part →					
	below middle		at middle		above middle	
width (ratio length/width)	 10 pyriform	 8 ovate	 (parallel) 5 cylindric	 (rounded) 6 elliptic	 9 obovate	 7 cordate
narrow (elongated)	 11 obcordate		 (parallel) 4 oblong	 (rounded) 3 circular		
			 2 oblate			
broad (compressed)			 1 flattened			

Ad. 29: Fruit: ribbing

Observation should be made at the pedicel end after removing the pedicel and calyx.

1
absent or very weak

3
weak

5
medium

7
strong

9
very strong

Ad. 30: Fruit: depression at pedicel end

1
absent or very weak

3
weak

5
medium

7
strong

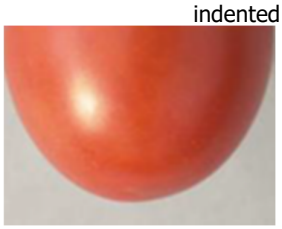
Ad. 31: Fruit: size of pedicel scar

Observations should be made on the green ring (not the full scar) after removal of the pedicel.

Ad. 33: Fruit: shape at blossom end

1
indented

2
indented to flat

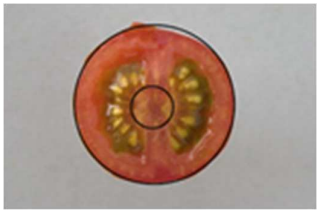


3
flat

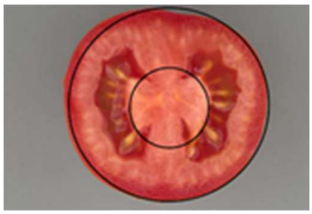
4
flat to pointed

5
pointed

Ad. 34: Fruit: diameter of core in cross section in relation to total diameter



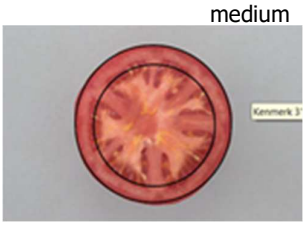
1
very small



3
small

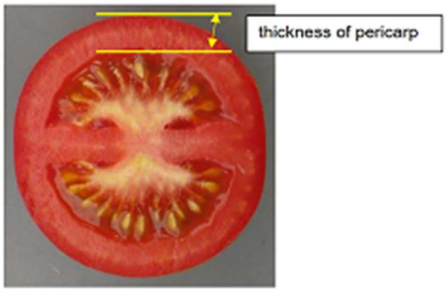
5
medium

7
large



9
very large

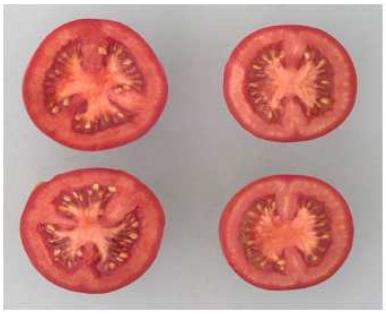
Ad. 35: Fruit: thickness of pericarp



Ad. 36: Fruit: number of locules

Observations should be made on cross sections of typical fruits, excluding the first and last fruits of the truss.

1
only two



2
two and three

3
three and four

4
four, five or six

5
more than six

Ad. 37: Fruit: gel in locules

1
absent

9
present

Ad. 38: Fruit: colour

Observations should be made when the colour has fully changed, and the placenta is visible in the cross section. Parent lines which do not ripen at all should be excluded.

Ad. 39: Fruit: colour of flesh

Parent lines which do not ripen at all should be excluded.

Ad. 40: Fruit: glossiness of skin

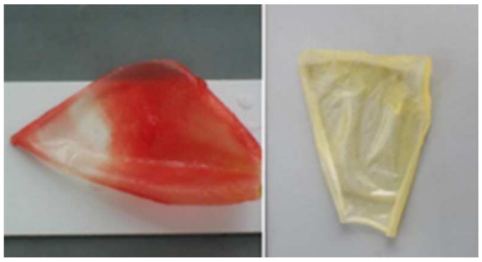
1
weak

2
medium

3
strong

Ad. 41: Fruit: colour of epidermis

The epidermis should be peeled off the fruit with a sharp knife. The fruit flesh may stick to the epidermis. Fruit flesh should be removed by scratching it delicately.



1
colourless

2
yellow

Ad. 42: Fruit: firmness

Observations should be made on completely coloured fruits. Firmness should be determined by hand on relation to example varieties.

Ad. 43: Time of flowering

The date of flowering is reached when 50% of plants have the third flower on the second truss open.

Ad. 44: Time of maturity

Time of maturity is reached when the first fruit on the second truss is fully ripe on 50% of plants.

Ad. 45: Resistance to *Meloidogyne incognita* (Mi)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Meloidogyne incognita*
2.	Quarantine status	-
3.	Host species	Tomato - *Solanum lycopersicum*
4.	Source of inoculum	GEVES[1] (F) or INIA (SP)[2] or Naktuinbouw (NL[3])
5.	Isolate	non-resistance breaking
6.	Establishment isolate identity	use tomato standards
7	Establishment pathogenicity	use susceptible rootstock or tomato standard
8.	Multiplication inoculum	
8.1	Multiplication medium	living plant
8.2	Multiplication variety	Susceptible variety, preferably resistant to powdery mildew
8.3	Plant stage at inoculation	2nd leaf stage
8.5	Inoculation method	deposit of piece of contaminated roots in soil (around 5-10g near each plant, to adapt depending of the population aggressivity)
8.6	Harvest of inoculum	6 at 10 weeks after inoculation, root systems are cut with scissors into pieces of about 1 cm length
8.7	Check of harvested inoculum	visual check for presence of root knots and ripe egg masses
8.8	Shelf life/viability inoculum	1 day
9.	Format of the test	
9.1	Number of plants per genotype	at least 30 plants, plus at least 10 non-inoculated plants to observe if a possible lack of germination is due to nematode or not. It is recommended to sow more seeds to be sure to get enough plants
9.2	Number of replicates	at least 2, preferably 3 replicates
9.3	Control varieties	ISF definitions: [4] Susceptible: Casaque Rouge Intermediate resistant (IR): Campeon and Tyonic Highly resistant (HR): Arletta, Anahu, Anahu x Casaque Rouge
9.4	Test design	3 replicates of 10 plants in different trays by variety, non-inoculated plants in separate tray
9.5	Test facility	greenhouse or climate room
9.6	Temperature	20-26°C, the temperature must be adapted depending on the aggressivity of the test to obtain expected response of controls but should not be above 26°C. Higher temperatures will cause breakdown of resistance.
9.7	Light	at least 12 h per day
10	Inoculation	
10.1	Preparation inoculum	small pieces of diseased roots mixed with soil
10.2	Quantification inoculum	the ratio is depending of aggressiveness of test and lab's conditions (e.g. between 30g to 60g of infested roots, for 100 plants in a tray of 45*30 cm containing approximately 5.5 kg of substrate), galls should be homogeneously mixed with soil.
10.3	Plant stage at inoculation	seed
10.4	Inoculation method	seeds sown in soil contaminated with galls
10.7	End of test	28 to 45 days after inoculation depending on test conditions (temperature, season)
11.	Observations	
11.1	Method	root inspection
11.2	Observation scale	

[1] GEVES; matref@geves.fr
[2] INIA; resistencias@inia.es
[3] Naktuinbouw; resistentie@naktuinbouw.nl
[4] ISF, https://www.worldseed.org

Class 0: healthy plant, no galls	Class 1: few and little galls which are difficult to find (for example less than 5)	Class 2: few galls, easy to observe but on few roots, still a lot of roots without galls	Class 3: many individual galls on most but not all roots	Class 4: many galls on all roots, sometimes in chains, can lead to dead plants and /or may suppress emergence
				

The germination percentage of non-inoculated plants of the same seed lot in the same experiment should be used to calculate the number of seeds that did not produce a plant due to the presence of nematodes and add these to plants in class 4.

11.3	Validation of test	Validation on controls. Expected reactions of controls: Susceptible control: - most plants at classes 3 and 4, - at most 2 plants can be observed at class 2. Intermediate resistant control: - clearly different from other controls, - with majority of plants around class 2. Highly resistant control: - most plants at classes 0 and 1, - at most 2 plants can be observed at class 2.
11.4	Off-types	Highly resistant varieties may have a few plants with a few galls
12.	Interpretation of data in terms of UPOV characteristic states	Resistance to *Meloidogyne incognita* (Mi): [1] absent or low: distribution of plants in the classes comparable with the susceptible controls. [2] medium: distribution of plants in the classes comparable with the intermediate resistant controls. [3] high: distribution of plants in the classes comparable with the highly resistant controls.
13.	Critical control points	Avoid overwatering. This may result in rotting of roots. In case of aggressive test, put seeds in a layer of non-contaminated soil or decrease the quantity of inoculum.

Ad. 46: Resistance to *Verticillium* sp. (Va and Vd) – Race 0

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Verticillium* sp. (see note below)
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[5] (NL) and GEVES[6] (FR)
5.	Isolate	Race 0 (e.g. isolate Toreilles 4-1-4-1)
6.	Establishment isolate identity	use differential varieties, see ISF website: https://www.worldseed.org
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar, Agar Medium "S" of Messiaen
8.4	Inoculation medium	water (for scraping agar plates) or Czapek Dox broth (3-7 d-old, aerated culture at 20-25°C, in darkness)
8.6	Harvest of inoculum	filter through double muslin cloth
8.7	Check of harvested inoculum	spore count; adjust to 10^6 per ml
8.8	Shelf life/viability inoculum	1 day at 4°C
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants, and at least 2 non-inoculated plants
9.3	Control varieties	
	Susceptible	Flix, Marmande verte, Moneymaker, Santonio
	Resistant	Monalbo, Marmande VR, "Monalbo x Marmande verte", Daniela, Elias
9.5	Test facility	greenhouse or climate room
9.6	Temperature	optimal 20-25°C, 20-22°C after inoculation
9.7	Light	12 h or longer
10.	Inoculation	
10.1	Preparation inoculum	aerated, liquid culture (8.4)
10.2	Quantification inoculum	count spores, adjust to 10^6 per ml
10.3	Plant stage at inoculation	cotyledon to 3rd leaf
10.4	Inoculation method	roots are immersed for 4 to 15 min in spore suspension
10.5	First observation	14 days after inoculation
10.7	Final observations	21 to 33 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	growth retardation, wilting, chlorosis, and vessel browning
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	All symptoms may be present in resistant varieties, but the severity will be distinctly less than in susceptible varieties. Usually, resistant varieties will show significantly less growth retardation than susceptible varieties. Observation of vessel browning is important for diagnosis. Usually, vessel browning will not extend to the 1st leaf in resistant varieties. Many hybrid varieties are heterozygous and appear to have mild symptoms in the biotest. Note: Resistance to *V. dahliae* based in the Ve gene is also effective to *V. albo-atrum*. Isolates of both fungal species may be used to evaluate the UPOV characteristic "Resistance to *V. dahliae*" or *V. albo-atrum* as long as the isolate belongs to the non-Ve breaking race 0. Resistance-breaking isolates have been described in both species.

[5] Naktuinbouw, resistentie@naktuinbouw.nl
[6] GEVES, matref@geves.fr

Ad. 47 + 48 + 49: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* - Race 0EU/1US (Fol: 0EU/1US), Race 1EU/2US (Fol: 1EU/2US) and Race 2EU/3US (Fol: 2EU/3US)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 1EU/2US and Race 2EU/3US to be tested in a bio-assay (method i) and/or in a DNA marker test (methods ii and iii). In case of a bio-assay, type of observation is VS/VG. In case of a DNA marker test, type of observation is MS.

(i) Bio-assay

1.	Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3.	Host species	*Solanum lycopersicum* L.
4.	Source of inoculum	GEVES[7] (FR), INIA-CSIC[8] (ES) or Naktuinbouw[9] (NL)
5.	Isolate	e.g. Reference strain validated in an interlaboratory test10. Race 0EU/1US (e.g. isolate Orange 71 or PRI 20698 or Fol 071), race 1EU/2US (e.g. isolate 4152, PRI40698 or RAF 70) and race 2EU/3US
6.	Establishment isolate identity	use differential varieties, see ISF website: https://www.worldseed.org
7	Establishment pathogenicity	on susceptible tomato varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar or Medium "S" of Messiaen or Czapek-Dox
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	see 10.2
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 plus at least 5 non-inoculated plants
9.2	Number of replicates	plants have to be divided into at least 2 replicates
9.3	Control varieties	
9.3.1	Control varieties for the test with race 0EU/1US	Susceptible: Marmande, Marmande verte, Resal, Moneymaker Resistant: Marporum, Larissa, "Marporum x Marmande verte", Motelle, Gourmet, and Riesling as additional resistant control for medium level
9.3.2	Control varieties for the test with race 1EU/2US	Susceptible: Marmande verte, Cherry Belle, Roma, Marporum, Ranco, Moneymaker Resistant: Tradiro, Motelle, "Motelle x Marmande verte", and Agostino as additional resistant control for medium level
9.3.3	Control varieties for the test with race 2EU/3US	Susceptible: Marmande verte, Motelle, Marporum Resistant: Alliance, Florida, Murdoch, "Marmande verte x Florida"
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
10	Inoculation	
10.1	Preparation inoculum	3-5 days in aerated liquid cultures like PDB, Czapek Dox or S of Messiaen or scraping of plates of 10 days cultures on agar medium.
10.2	Quantification inoculum	spore count, adjust to 10^6 spores per ml, in case of very aggressive isolate inoculum concentration can be decreased
10.3	Plant stage at inoculation	10-18 d, cotyledon to first leaf
10.4	Inoculation method	plants at the inoculation stage are harvested carefully, roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option, and transplanted in trays
10.7	End of test	14-21 days after inoculation

[7] GEVES: matref@geves.fr
[8] INIA: resistencias@inia.es
[9] Naktuinbouw: resistentie@naktuinbouw.nl
[10] Harmores 3 CPVO project: https://cpvo.europa.eu/sites/default/files/documents/report_harmores_3_final_meeting_v0_0.pdf

11.	Observations	
11.1	Method	visual
11.2	Observation scale	

Class 0	Class 1	Class 2	Class 3
Healthy compared to the non-inoculated control.	Healthy compared to the non-inoculated control with brown vessel above the cotyledon (observed when plants are cut in case of variety with different levels of symptoms)	Higher than 50% of growth reduction and/or yellowing and/or wilting on cotyledons and/or leaves.	Nearly dead: strong reduction with plants look dwarf (there can be necrosis but not always) or dead
			
If all plants in class 0 or if all plants in classes 2 and 3, it is not necessary to cut the plants.			
In case of variety or control with different levels of symptoms, cut the plants to check presence or not of strong brown vessel above cotyledons. In case of no brown vessels or below cotyledons, the plant is note 0. In case of brown vessels above cotyledons, the plant is note 1.			

11.3	Validation of test	Validation on controls. Expected response of controls: Susceptible control: - most plants in 2 and 3, at most 10% of the plants class 0 and 1 Resistant control: - most plants in 0 and 1, at most 10% of the plants class 2 and 3. Controls with medium level of resistance can show a higher number of plants in class 2 and 3.
12.	Interpretation of data in terms of UPOV characteristic states	[1] absent: Average symptom level higher than in the medium-resistant control [9] present: Average symptom level not different from the medium-resistant control or the high-resistant control

(ii) DNA marker test on gene *I-2*

The resistance gene *I-2* confers resistance to both *Fusarium oxysporum* f. sp. *lycopersici* Fol:1(EU)/2(US) and Fol:0(EU)/1(US). The presence of the resistant allele and/or the susceptible allele can be detected by the co-dominant TaqMan marker based on the dominant marker described in Arens et al. (2010).

Specific aspects:

1.	Pathogen	*Fusarium oxysporum* f.sp. *lycopersici Fol: 1(EU)/2(US)*
2.	Functional gene	*I-2*
3.	Primers	
3.1	universal primers	
	I2 Forward Primer	5'-AATGATGAGAGRGTGAAGAAWCA-3'
	I2 Reverse Primer	5'-TCTTTCCCTTCAAACTTTCCTTCA-3'
3.2	Allele specific probes	Recommended probes are MGB probes (Applied biosystems) or XS probes (Biolegio) the Tm of the probes must be ordered at 68°C.
	Susceptible i2 probe	5'-6FAM*-TTGACAGCTTGGTTTTGT-BHQ1-3'
	Resistance I2 probe	5'-TEXAS RED*-TTTGAAAGCGTGGTATTGC-BHQ2-3'
		*Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine.
4.	Format of the test	
4.1	Number of plants per genotype	At least 20 plants (individual DNA extraction and PCR for each plant)
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 (for example commercial kit for plant DNA extraction, or lab prepared reagents)
5.2	Preparation PCR	Pipette each DNA sample and a commercial real-time PCR mastermix into individual wells. Analyze the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction

		conditions suitable for the mastermix used. For this test the PerfeCta Multiplex qPCR Toughmix (Quantabio) is commonly used.
5.3	example PCR mastermix	

	Initial concentration	Volume/ reaction (µL)	Final concentration
PerfeCta Multiplex qPCR Toughmix	5x	4	1X
FW primer	10µm	0.75	375nM
Rev primer	10µm	0.75	375nM
Vat-pr (Fam)	10µm	0.3	150nM
Res-pr (TR)	10µm	1.3	650nM
H_2O	-	9.9	-
Subtotal		17	-
DNA		3	-
Total		20	-

6.	PCR conditions	
	1.	Initial denaturation step at 94°C for 2-10 minutes (mastermix dependent)
	2.	40 cycles at 94°C for 15 sec, 60°C 1 min. Every cycle ends with plate reading
	3.	Analysis of Ct values for each probe is done to identify positive (+) reactions at Ct<35, or negative reactions (no Ct value). Reactions with Ct values 35-40 should be repeated. Analysis can also be done with a genotyping end point fluorescence reading
7.	Validity and Interpretation of Results	
7.1	Validity of the result	• Check for typical exponential amplification curves for each sample, as expected for normal specific amplification.
		• Non-specific amplification is possible in a PCR reaction. Check the results for the presence of non-exponential curves and/or curves just above the threshold. These curves should be assessed as negative.
		• Check if the control samples are as expected (negative control = no signal; positive controls = show signals for all fluorophores).
7.2	Interpretation of the results	• Ct values can be determined using for example a set threshold (*single threshold*) of 200 RFU for each of the fluorescence labels.
		• For low or high Ct values the DNA concentration should be checked. If the DNA concentration is low, high Ct values are expected. For samples with a high DNA concentration, low Ct values are expected. If two fluorophores are present, both fluorophores will show the high or the low Ct value.
	Decision Matrix	

Signal specific Fluorophore*				
Fam Susceptible i-2**	Texas Red Resistance I-2 **	**Molecular Interpretation**	**Conclusion regarding resistance to FoI: 1(EU)/2(US)**	**Control variety**
+	-	i-2/i-2	Absent***	Marmande Verte
+	+	I-2/i-2	Present	Motelle x Marmande Verte
-	+	I-2/I-2	Present	Tradiro
-	-	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		

* + signal is above the threshold and curves are as expected; - signal is not above the threshold or curves are non-exponential.
** Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine.
*** Susceptible, or possibly resistant on another mechanism like gene *I-3*

8.	Conclusion	A conclusion of presence/absence of resistance should be made for each variety based on the results of the 20 individual plant genotypes. A tolerance of 1 individual out of type plant can be made, otherwise the variety should be identified as heterogenous if contradictory results are obtained for a variety

This protocol was validated by a ring-test with three different laboratories (Interlaboratory Comparative Test Report, INVITE 2023). If a different protocol is used, the laboratory must validate its method in comparison to the reference method to show that the alternative protocol gives the same results. Validation of an alternative test should be carried on at least 50 varieties, with 12 individuals per variety. The varieties should cover all available outcomes as evenly as possible. After validation, the report and results should be peer-reviewed by one (preferably 2) of the EO's performing resistance testing.

(iii) DNA marker test on gene *I-3*

The resistance gene *I-3* confers resistance to *Fusarium oxysporum* f. sp. *lycopersici* Fol:0(EU)/1(US), Fol:1(EU)/2(US) and Fol:2(EU)/3(US).
The presence of the resistant allele and/or the susceptible allele can be detected by the co-dominant TaqMan marker based on the CAPS marker described in Gonzalez-Cendales et al, 2014.

Specific aspects: *Fusarium oxysporum* f.sp. *lycopersici* Fol: 2(EU)/3(US)

1	Characteristic	*Fusarium oxysporum* f.sp. l*ycopersici* Fol: 2(EU)/3(US)
2	Genes and alleles	*I-3*
2.1	Targeted gene(s)	Resistance allele *I-3* Genbank KP082943.1: SpSRLK-5 Genbank HG975446.1 *Solanum penellii* Chromosome 7 Susceptible allele/ homologs *i-3* Genbank HG975519.1 chromosome 7 variety M82 SpSRLK-6: genbank KP082944.1
		Gonzalez-Cendales et al, 2014. Catanzariti 2015
2.2	Allele corresponding to expression state 1	Susceptible gene/ homologs *i-3* chromosome 7 variety M82: Genbank HG975519.1 SpSRLK-6: genbank KP082944.1
2.3	Allele corresponding to expression state 9	Resistance Gene *I-3* SpSRLK-5: Genbank KP082943.1 Genbank HG975446.1 *Solanum penellii* Chromosome 7 Gonzalez-Cendales et al, 2014. Catanzariti 2015
3	Primers (and probes)	
3.1	Primers to detect both alleles	Forward Primer: 5'-CAATGGTCTGTAGTTGATTGGAATG-3' Reverse Primer: 5'-CTGCCCAAGCCACAATTTAG-3'
3.2	Probes to detect both alleles	Susceptible *i-3* probe: 5'-6-FAM-TGTACGAATAATGGGC-MGB-NFQ-3' Resistance *I-3* probe: 5'-VIC-TTGTACAAATAACGGGC-MGB-NFQ-3' The used probes for the design are MGB probes (Applied biosystems).
4	Format of the test	
4.1	Number of plants per genotype	20 plants (individual DNA extraction and PCR for each plant)
4.2	Control varieties	
4.3	Process controls	Negative control (H2O), positive control (sample containing the expected alleles)
5	Preparations	

5.1	Preparation DNA	Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol (for example commercial kit for plant DNA extraction, or lab prepared reagents)
5.2	Preparation PCR	Pipette each DNA sample and a commercial real-time PCR mastermix into individual wells. Analyze the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction conditions suitable for the mastermix used. The following instructions (5.3) relate to the use of the Quanta Perfecta Multiplex qPCR Toughmix. However, other mastermix suitable for the combination of probes and primers may also be used.
5.3	Example PCR mastermix	

	Initial concentration	**Volume/ reaction (µL)**	**Final concentration**
Quanta Perfecta multiplex toughmix	5x	4	1X
Forward Primer	10µm	0.75	375nM
Reverse Primer	10µm	0.75	375nM
Probe2-*i-3*-Susceptible (Fam)	10µm	0.2	100nM
Probe2-*I-3*-Resistant (VIC)	10µm	0.3	150nM
H_2O	-	11	-
subtotal		*17*	-
DNA (5-10ng/µl)		3	-
Total		**20**	-

6	Technique of the method	
6.1	Particular conditions	PCR conditions: 1. Initial denaturation step at 94°C for 2-10 minutes (mastermix dependent) 2. 40 cycles at 94°C for 15 sec, 60°C 1 min. Every cycle ends with plate reading 3. Analysis of Ct values for each probe is done to identify positive (+) reactions at Ct<35, or negative reactions (no Ct value). This cut-off may need to be adapted to laboratory equipment and reagent and must be validated by each laboratory using controls. Reactions with Ct values 35-40 should be repeated. Analysis can also be done with a genotyping end point fluorescence reading.
7	Observations	
7.1	Validity of the results	•Check for typical exponential amplification curves for each sample, as expected for normal specific amplification. •Non-specific amplification is possible in a PCR reaction. Check the results for the presence of non-exponential curves and/or curves just above the threshold. These curves should be assessed as negative. •Check if the control samples are as expected (negative control: no signal; positive controls: shows expected signals for the fluorophores).

8	Interpretation of the test results	•Ct values are determined using a set threshold (single threshold) of 200 RFU for each of the fluorescence labels, this value may need to be adapted to each machine. •For low or high Ct values the DNA concentration should be checked. If the DNA concentration is low, high Ct values are expected. For samples with a high DNA concentration, low Ct values are expected. If two fluorophores are present, both fluorophores will show the high or the low Ct value. •In case the DNA marker test result does not confirm the declaration in the Technical Questionnaire, a bio-assay should be performed.
8.1	Decision Matrix:	

Signal specific Fluorophore*				
Fam Susceptible *i-3***	VIC *I-3*** Resistant	**Molecular Interpretation**	**Conclusion regarding resistance to Fol: 2(EU)/3(US)**	**Control variety in this test**
+	-	*i-3*/*i-3*	Absent***	Marmande verte, Motelle, Marporum
+	+	*i-3*/*I-3*	Present	Strongton
-	+	*I-3*/*I-3*	Present	Zerozerosette
-	-	Invalid result. Repeat assay or bio assay should be performed.		

* + signal is above the threshold and curves are as expected; - signal is not above the threshold or curves are non-exponential.
** Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine.
*** Susceptible, or possibly resistant on another mechanism like gene *I-7*

9	Validation of the method	A conclusion of presence/absence of resistance should be made for each variety based on the results of the 20 individual plant genotypes. A tolerance of 1 individual out of type plant can be made, otherwise the variety should be identified as heterogeneous if contradictory results are obtained for a variety. If a different protocol is used, the laboratory must validate its method in comparison to the reference method to show that the alternative protocol gives the same results.
9.1	Contact Examination Office	Naktuinbouw
10	References	Gonzalez-Cendales Y., Do H.T.T., Lim G.T.T., McGrath D. J., Catanzariti A.M., Jones D.A. 2014. Application of CAPS Markers to the Mapping and Marker-Assisted Breeding of Genes for Resistance to Fusarium Wilt in the Tomato. ISBN: 978-1-63117-553-4, Chapter 6 Nova Science Publishers, Inc. Catanzariti A.M. Lim G.T.T., Jones D.A., 2015. The tomato *I-3* gene: a novel gene for resistance to Fusarium wilt disease. New Phytologist (2015) 207: 106–118

Ad. 50: Resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* (For)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Fusarium oxysporum* f. sp. *radicis-lycopersici*
2.	Quarantine status	
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[11] (NL) and GEVES[12] (FR)
5.	Isolate	-
7.	Establishment pathogenicity	symptoms on susceptible tomato
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar, or Medium agar "S" of Messiaen
8.4	Inoculation medium	Water for scraping agar plates or Czapek-Dox (7 d-old aerated culture)
8.6	Harvest of inoculum	filter through double muslin cloth
8.7	Check of harvested inoculum	spore count; adjust to 10^6 per ml
8.8	Shelflife/viability inoculum	4-8 h, keep cool to prevent spore germination
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	Not applicable
9.3	Control varieties	
	Susceptible	Motelle, Moneymaker
	Resistant	Momor, "Momor x Motelle"
	Remark	"Momor x Motelle" has slightly weaker resistance than Momor
9.4	Test design	>20 plants, e.g. 35 seeds for 24 plants, including 2 non-inoculated controls
9.5	Test facility	glasshouse or climate room
9.6	Temperature	24-28°C (severe test, with mild isolate) 17-24°C (mild test, with severe isolate)
9.7	Light	at least 12 hours per day
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 culture or scraping of plates
10.2	Quantification inoculum	spore count, adjust to 10^6 spores per ml
10.3	Plant stage at inoculation	12-18 d, cotyledon to third leaf
10.4	Inoculation method	roots and hypocotyls are immersed in spore suspension for 5-15 min
10.7	Final observations	10-21 days after inoculation
11.	Observations	
11.1	Method	visual; a few plants are lifted at the end of the test
11.2	Observation scale	Symptoms: Plant death Growth retardation caused by root degradation Root degradation Necrotic pinpoints and necrotic lesions on stems
11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
11.4	Off-types	
12.	Interpretation of data in terms of UPOV characteristic states	absent [1] symptoms present [9] no symptoms
13.	Critical control points	Temperature should never exceed 27°C during the test period. Isolates may lose pathogenicity after repeated subculturing. Isolates should not be subcultured more than two times.

[11] Naktuinbouw, resistentie@naktuinbouw.nl
[12] GEVES, matref@geves.fr

Ad. 51 + 52 + 53 + 54 + 55 + 56 + 57 + 58 + 59: Resistance to *Passalora fulva* (Pf) – Race 0, Race A, Race B, Race C, Race D, Race E, Race F, Race H, and Race J

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

Resistance to *Passalora fulva* (Pf) to be tested in a bio-assay (method i) and/or in a DNA marker test (methods ii and iii). In case of a bio-assay, type of observation is VS/VG. In case of a DNA marker test, type of observation is MS.

(i) bio-assay

1.	Pathogen	*Passalora fulva*
2.	Quarantine status	-
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[13] (NL) or GEVES[14] (FR)
5.	Isolate	Races 0, A, B, C, D, E, F, H and J
6.	Establishment isolate identity	with genetically defined differentials A breaks *Cf-2*, B *Cf-4*, C *Cf-2* and *Cf-4*, D *Cf-5*, E *Cf-2*, *Cf-4* and *Cf-5*, F *Cf-2* and *Cf-9*, H *Cf-4* and *Cf-9*, J *Cf-2, Cf-6* and *Cf-9* https://www.worldseed.org
7.	Establishment pathogenicity	symptoms on susceptible tomato
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar or Malt Agar or a synthetic medium
8.8	Shelflife/viability inoculum	4 hours, keep cool
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Monalbo, Moneymaker
	Resistant for Race A:	Purdue, IVT1154, IVT1149, Antique, Pink Treat, Sprigel, Triatlon
	Resistant for Race B:	Vétomold, IVT1154, IVT1149, Antique, Retinto, Sprigel, Triatlon
	Resistant for Race C:	IVT1154, IVT1149, Antique, Sprigel, Triatlon
	Resistant for Race D:	Vétomold, IVT1154, Antique, Pink Treat, Retinto, Sprigel
	Resistant for Race E:	IVT 1154, Antique, Sprigel
	Resistant for Race F: Resistant for Race H:	Purdue 135, IVT1149, Ontario 7818, Chelino, Completo Vétomold, IVT1149, Ontario 7818, Chelino, Completo
	Resistant for Race J:	Purdue 135, IVT1149
9.5	Test facility	glasshouse or climate room
9.6	Temperature	day: 22° C, night: 20°or day: 25°C, night 20°C
9.7	Light	12 hours or longer
9.8	Season	
9.9	Special measures	depending on facility and weather, there may be a need to raise the humidity, e.g. humidity tent fully closed 3-4 days after inoculation and after that partly closed (66% to 80%, 24 h per day), until end
10.	Inoculation	
10.1	Preparation inoculum	prepare evenly colonized plates, e.g. 1 for 36 plants; remove spores from plate by scraping with water with Tween20; filter through double muslin cloth
10.2	Quantification inoculum	count spores; adjust to 10^5 spores per ml or more
10.3	Plant stage at inoculation	19-20 d (incl. 12 d at 24°), 2-3 leaves
10.4	Inoculation method	spray on dry leaves
10.7	Final observations	14 days after inoculation; when susceptible control does not show clear symptoms the test may be prolonged until for example 18 days after inoculation
11.	Observations	
11.1	Method	visual inspection of abaxial side of inoculated leaves
11.2	Observation scale	Symptom: velvety, white spots
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls

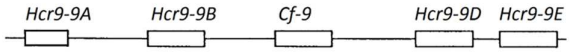
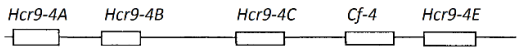
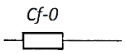
[13] Naktuinbouw; resistentie@naktuinbouw.nl
[14] GEVES; matref@geves.fr

12.	Interpretation of data in terms of UPOV characteristic states	absent [1] symptoms present [9] no symptoms
13.	Critical control points	Pf spores have a variable size and morphology. Small spores are also viable. Fungal plates will gradually become sterile after 6-10 weeks and repeated subculturing. Do not subculture more often than strictly necessary for multiplication. Excessively high humidity may cause rugged brown spots on all leaves.

(ii) DNA marker test on genes *Cf-4* and *Cf-9*

The resistance gene *Cf-9* confers resistance to *Passalora fulva* (Pf) - Races 0, A, B, C, D and E. The resistance gene *Cf-4* confers resistance to *Passalora fulva* (Pf) Races 0, A, D, F, G and J.
The presence of the resistant allele can be detected by the multiplex TaqMan marker based on the assay developed on the data described by Kim et al., 2016

Specific aspects:

1	Characteristic	*Passalora Fulva* (Pf)
2	Genes and alleles	*Cf-9 and Cf-4*
2.1	Targeted gene(s)	Resistance Gene *Cf-9* Genbank AJ002236*: Lycopersicon pimpinellifolium Cf-9* resistance gene cluster.  Resistance Gene *Cf-4* GenBank: AJ002235.1: *Lycopersicon hirsutum Cf-4* resistance gene cluster  Susceptible allele GenBank: AJ002237.1: *Lycopersicon esculentum* haplotype of the *Cf-4*/9 resistance gene locus  Homologous HCR9-9A; HCR9-9B; HCR9-9D; HCR9-9E HCR9-4A; HCR9-4B; HCR9-4C; HCR9-4E LAT (Linker For Activation Of T Cells) is used as an internal amplification control. Some susceptible varieties show an allele that differs from the *Cf-0* alleles. Kim et al., 2017
2.2	Allele corresponding to expression state 1	Susceptible allele GenBank: AJ002237.1: *Lycopersicon esculentum* haplotype of the *Cf-4*/9 resistance gene locus
2.3	Allele corresponding to expression state 9	Resistance Gene Resistance Gene *Cf-9* Genbank AJ002236*: Lycopersicon pimpinellifolium Cf-9* resistance gene cluster. Resistance Gene *Cf-4* GenBank: AJ002235.1: *Lycopersicon hirsutum Cf-4* resistance gene cluster Kim et al., 2016
3	Primers (and probes)	

3.1	Primers to detect alleles	Forward Primers: *Cf-0* TqMn F1 5'-GGTTATCACTTGGAAATAACAACTTAC-3' *Cf-4* TqMn F1 5'-ATCTTGAAGGACCAATTCCAT-3' *Cf-9* F2 5'-CTATGGAATCTCACCAACATAGTGTT-3' Lat Fw 5'-AGACCACGAGAACGATATTTGC-3' Reverse Primers: *Cf-0* TqMn R1 5'-GGTTTGATGACAAGAAGAGCC-3' *Cf-4* TqMn R1 5'-GCTCAAGTCTAACCCTATCAGTGAT-3' *Cf-9* R2 5'-CCGTTCAAGTTGGGTGTTAA-3' Lat Rev 5'-CTTGCCTTTTCATATCCAGACA-3'
3.2	Probes to detect both alleles	*Cf-0* TqMn probe1* Tm 62,9°C 5'-6Fam-ATAATATTTCAAGCTGGGTCCAGCTTCTGTT-BHQ1-3' *Cf-4* TqMn Probe1* Tm 61,6°C 5'-Vic-ACCAAAGTATTTGTAGGTTACGTAGTCCGC-BHQ1-3' *Cf-9* TqMn Probe1* Tm 60,8°C 5'-Texas Red-TTTCACGATATTTGAAAAGCTCAAGAGGTT-BHQ2-3' Lat Probe* TM 61,8°C 5'-Cy5-AGTTGTGAAAAGCCCAAGGGAGGACT-BHQ2-3' *Fluorophores and quenchers can be modified according to compatibility with the filters on the real-time PCR machine.
4	Format of the test	
4.1	Number of plants per genotype	20 plants (individual DNA extraction and PCR for each plant)
4.2	Control varieties	
4.3	Process controls	Negative control (H2O), positive control (sample containing the expected alleles)
5	Preparations	
5.1	Preparation DNA	Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol (for example commercial kit for plant DNA extraction, or lab prepared reagents)
5.2	Preparation PCR	Pipette each DNA sample and a commercial real-time PCR master mix into individual wells. Analyse the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction conditions suitable for the master mix used. The following instructions relate to the use of the TaqMan gene expression master mix of Applied Biosystems. However, other master mix suitable for the combination of probes and primers may also be used.
5.3	Example PCR mastermix	

		Initial Concentration	**1x reaction**		**Final reaction concentration**
	Tagman gene expression master mix	2X	12,5	µl	1X
	Cf-0 TqMn F1	10µm	0,75	µl	300nM
	Cf-0 TqMn R1	10µm	0,75	µl	300nM
	Cf-0 TqMn probe1	10µm	0,5	µl	200nM
	Cf-4 TqMn F1	10µm	0,75	µl	300nM
	Cf-4 TqMn R1	10µm	0,75	µl	300nM
	Cf-4 TqMn Probe1	10µm	0,5	µl	200nM
	Cf-9 F2	10µm	0,75	µl	300nM
	Cf-9 R2	10µm	0,75	µl	300nM
	Cf-9 TqMn Probe1	10µm	0,5	µl	200nM
	Lat Fw	10µm	0,75	µl	300nM
	Lat Rev	10µm	0,75	µl	300nM
	Lat Probe	10µm	0.5	µl	200nM
	H2O	10µm	1,5	µl	
	Subtotal	-	22	µl	
	DNA Sample	5-10ng/ul	3	µl	
	Total		25	µl	

6	Technique of the method	
6.1	Particular conditions	PCR conditions: 1. Initial denaturation step at 94°C for 2-10 minutes (mastermix dependent) 2. 40 cycles at 94°C for 15 sec, 60°C 1 min. Every cycle ends with plate reading 3. Analysis of Ct values for each probe is done to identify positive (+) reactions at Ct<35, or negative reactions (no Ct value). This cut-off may need to be adapted to laboratory equipment and reagent and must be validated by each laboratory using controls. Reactions with Ct values 35-40 should be repeated. Analysis can also be done with a genotyping end point fluorescence reading.
	Observations	
7.1	Validity of the results	•Check for typical exponential amplification curves for each sample, as expected for normal specific amplification. •Non-specific amplification is possible in a PCR reaction. Check the results for the presence of non-exponential curves and/or curves just above the threshold. These curves should be assessed as negative. •Check if the control samples are as expected (negative control: no signal; positive controls: shows expected signals for the fluorophores).
8	Interpretation of the test results	•Ct values are determined using a set threshold (single threshold) of 200 RFU for each of the fluorescence labels, this value may need to be adapted to each machine. •For low or high Ct values the DNA concentration should be checked. If the DNA concentration is low, high Ct values are expected. For samples with a high DNA concentration, low Ct values are expected. If two fluorophores are present, both fluorophores will show the high or the low Ct value. •In case the DNA marker test result does not confirm the declaration in the Technical Questionnaire, a bio-assay should be performed.
8.1	Decision Matrix:	

Fluorophores*

Fam ** *Cf-0* Susceptible	Vic ** *Cf-4* Resistant	Texas Red** *Cf-9* Resistant	Cy5** LAT control	Test conclusion	Molecular conclusion	Molecular interpretation	Control varieties
+	-	-	+/-	Valid result	*Cf-4* and *Cf-9* absent	Susceptible***	Monalbo
+	+	-	+/-	Valid result	*Cf-4* present	Races 0, A, D, F, G, J resistant ****	Purdue 135, Pink Treat
+	-	+	+/-	Valid result	*Cf-9* present	Races 0, A, B, C, D, E resistant ****	IVT 1154, Sprigel
-	+	-	+/-	Valid result	*Cf-4* present	Races 0, A, D, F, G, J resistant ****	Purdue 135
-	+	+	+/-	Valid result	*Cf-4* and *Cf-9* present	Races 0, A, B, C, D, E, F, G, J resistant	
-	-	+	+/-	Valid result	*Cf-9* present	Races 0, A, B, C, D, E resistant	IVT 1154
-	-	-	+	Valid result	Alternative allele is present ***	bioassay	
+	+	+	+/-	invalid result	Repeat or bioassay	Repeat or bioassay	
-	-	-	-	invalid result			

* + : signal is above the threshold and curves are as expected;
– : signal is not above the threshold or curves are non-exponential.
**: Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine.
***: Susceptible, or possibly resistant on another mechanism
****: see ISF report https://worldseed.org/document/isf-passalora-fulva-project-tomato-final-report-2019-2022/
+/- : + or – score, does not influence the outcome.

9	Validation of the method	A conclusion of presence/absence of resistance should be made for each variety based on the results of the 20 individual plant genotypes. A tolerance of 1 individual out of type plant can be made, otherwise the variety should be identified as heterogeneous if contradictory results are obtained for a variety. If a different protocol is used, the laboratory must validate its method in comparison to the reference method to show that the alternative protocol gives the same results.
9.1	Contact Examination Office	Naktuinbouw
10	References	Kim B., Hwang I. S., Lee H.J., Oh C.S., 2017. Combination of newly developed SNP and InDel markers for genotyping the *Cf-9* locus conferring disease resistance to leaf mold disease in the tomato. Mol Breeding (2017) 37: 59 DOI 10.1007/s11032-017-0663-3

(iii) DNA marker test on gene *Cf-5*

The resistance gene Cf-5 confers resistance to *Passalora fulva* (Pf) - Races 0, A, B, C, F, G, H and J. The presence of the resistant allele can be detected by the TaqMan marker based on the assay developed on the data described by Dixon M.S (1998).

Specific aspects:

1	Characteristic	*Passalora fulva* (Pf)
2	Genes and alleles	*Cf-5*

2.1	Targeted gene(s)	Resistance Gene *Cf-5* AF053993.1; Lycopersicon esculentum disease resistance protein (*Cf-5*) gene 
		As an internal control a marker for Linker For Activation Of T Cells (LAT) is included
		Dixon et al, 1998
2.2	Allele corresponding to expression state 1	The SNP in the *Cf-5* gene that causes a functional loss of the gene, is present in the homologous in a repeated fashion, causing a dominant signal.
2.3	Allele corresponding to expression state 9	Resistance Gene AF053993.1; *Lycopersicon esculentum* disease resistance protein (*Cf-5* gene) Dixon et al, 1998
3	Primers (and probes)	
3.1	Primers to detect resistant allele and internal control	Forward Primers: • *Cf-5* FW 5'-TTGGGTGAGAATGCTCTTAATGG-3' • Lat FW 5'-AGACCACGAGAACGATATTTGC-3' Reverse Primers: • *Cf-5* Rev 5'-TTGTAAAGATCCAACCTAGACAAGTTG-3' • Lat Rev 5'-CTTGCCTTTTCATATCCAGACA-3'
3.2	Probes to detect resistant allele and internal control	*Cf-5* Probe TM 68°C 5'-6FAM-ATTCCTTCTTCATTG-BHQ1 Lat Probe TM 61,8°C 5'-Cy5-AGTTGTGAAAAGCCCAAGGGAGGACT-BHQ2-3' Recommended probes are XS probes the Tm of the XS probes must be ordered at 68°C. MGB probes do not gain the temperature that is necessary for the assay. Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine. LAT (Linker For Activation Of T Cells) is used as an internal amplification control.
4	Format of the test	
4.1	Number of plants per genotype	20 plants (individual DNA extraction and PCR for each plant)
4.2	Control varieties	1: Moneymaker 9: Triatlon
4.3	Process controls	Negative control (H2O), positive control (sample containing the expected alleles)
5	Preparations	
5.1	Preparation DNA	Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol (for example commercial kit for plant DNA extraction, or lab prepared reagents)
5.2	Preparation PCR	Pipette each DNA sample and a commercial real-time PCR mastermix into individual wells. Analyse the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction conditions suitable for the mastermix used. The following instructions relate to the use of the TaqMan gene expression master mix from Applied Biosystems. However, other mastermix suitable for the combination of probes and primers may also be used.
5.3	Example PCR mastermix	

			Initial Concentration	**1x reaction**	**Final concentration**
		Taqman gene expression master mix	2X	12,5µl	1X
		Cf-5 Fw	10µm	1µl	400nM
		Cf-5 Rev	10µm	1µl	400nM
		Cf-5 Probe	10µm	0,75µl	300nM
		Lat Fw	10µm	0,5µl	200nM
		Lat Rev	10µm	0,5µl	200nM
		Lat Probe	10µm	0,3µl	120nM
		H2O		5,45µl	
		Subtotal		22µl	
		Sample 5-10ng/ul		3µl	
		Total		25µl	
6	Technique of the method				
6.1	Particular conditions	PCR conditions: 1. Initial denaturation step at 94°C for 2-10 minutes (mastermix dependent) 2. 40 cycles at 94°C for 15 sec, 60°C 1 min. Every cycle ends with plate reading 3. Analysis of Ct values for each probe is done to identify positive (+) reactions at Ct<35, or negative reactions (no Ct value). This cut-off may need to be adapted to laboratory equipment and reagent and must be validated by each laboratory using controls. Reactions with Ct values 35-40 should be repeated. Analysis can also be done with a genotyping end point fluorescence reading.			
7	Observations				
7.1	Validity of the results	•Check for typical exponential amplification curves for each sample, as expected for normal specific amplification. •Non-specific amplification is possible in a PCR reaction. Check the results for the presence of non-exponential curves and/or curves just above the threshold. These curves should be assessed as negative. •Check if the control samples are as expected (negative control: no signal; positive controls: shows expected signals for the fluorophores).			
8	Interpretation of the test results	•Ct values are determined using a set threshold (single threshold) of 200 RFU for each of the fluorescence labels, this value may need to be adapted to each machine. •For low or high Ct values the DNA concentration should be checked. If the DNA concentration is low, high Ct values are expected. For samples with a high DNA concentration, low Ct values are expected. If two fluorophores are present, both fluorophores will show the high or the low Ct value. •In case the DNA marker test result does not confirm the declaration in the Technical Questionnaire, a bio-assay should be performed.			
8.1	Decision Matrix:				

Fluorophores*					
Fam ** *Cf-5* Resistant	Cy5** LAT Control	Test conclusion	Molecular conclusion	Molecular interpretation	control variety
+	+/-	valid result	*Cf-5* Gene present	*Cf-5* Resistant (races 0,A,B,C,F,G,H,J)***	IVT 1149, Triatlon
-	+	valid result	*Cf-5* Gene absent	Susceptible, or resistant by another mechanism	Monalbo
-	-	invalid result	Repeat/or Bioassay	Repeat/or Bioassay	

* + signal is above the threshold and curves are as expected; - signal is not above the threshold or curves are non-exponential.
** Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine.
***See ISF report: https://worldseed.org/document/isf-passalora-fulva-project-tomato-final-report-2019-2022/

9	Validation of the method	A conclusion of presence/absence of resistance should be made for each variety based on the results of the 20 individual plant genotypes. A tolerance of 1 individual out of type plant can be made, otherwise the variety should be identified as heterogeneous if contradictory results are obtained for a variety. If a different protocol is used, the laboratory must validate its method in comparison to the reference method to show that the alternative protocol gives the same results.
9.1	Contact Examination Office	Naktuinbouw
10	Reference	M. S. Dixon, K. Hatzixanthis, D. A. Jones, K. Harrison, and J. D. Jones, "The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number," Plant Cell, vol. 10, no. 11, pp. 1915–1925, 1998

Ad. 60 + 61 + 62: Resistance to Tomato mosaic virus - Strain 0 (ToMV: 0), strain 1 (ToMV: 1), and strain 2 (ToMV: 2)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

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), if appropriate.

(i) Bio-assay

1.	Pathogen	Tomato mosaic virus
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[15] (NL) or GEVES[16] (FR) or INIA - CSIC[17] (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 standards Mobaci (Tm1), Moperou (Tm2), Momor (Tm2^2) Use diffential varieties, see ISF website: https:// www.woldseed.org
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

[15] Naktuinbouw, resistentie@naktuinbouw.nl
[16] GEVES, matref@geves.fr
[17] INIA – CSIC, resistencias@inia.es

9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Marmande, Monalbo, Moneymaker
	Resistant to ToMV: 0 and 2	Mobaci
	Resistant to ToMV: 0 and 1	Moperou
	Resistant to ToMV: 0, 1 and 2	"Monalbo x Momor" (with necrosis), Gourmet, Mocimor, Momor
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/30 ml)
10.4	Inoculation method	gentle rubbing
10.6	Second observation	cotyledons or 2 leaves
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 data in terms of UPOV characteristic states	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. Remark: Strain INRA Avignon 6-5-1-1 is recommended for ToMV: 0. This strain causes a striking yellow Aucuba mosaic.

(ii) DNA marker test

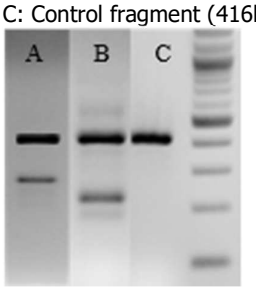
Resistance to ToMV is often based on resistance gene Tm2 (allele Tm2 or $Tm2^2$). The presence of the resistant alleles Tm2 and $Tm2^2$ and/or susceptible allele tm2 can be detected by the co-dominant markers as described in Arens, P. *et al* (2010). Two methods are available, conventional PCR and Taqman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

(a) Conventional PCR

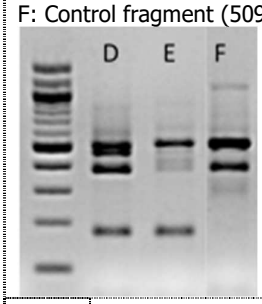
1.	Pathogen	Tomato mosaic virus
2.	Functional gene	$Tm2/2^2$ (with two alleles for resistance Tm2 and $Tm2^2$ and one allele for susceptibility tm2)
3.	Primers	
3.1	Assay 1 to check resistant allele Tm2 or $Tm2^2$	Outer primer TMV-2286F: 5'GGGTATACTGGGAGTGTCCAATTC3' Outer primer TMV-2658R: 5'CCGTGCACGTTACTTCAGACAA3' $Tm2^2$ SNP2494F: 5'CTCATCAAGCTTACTCTAGCCTACTTTAGT3' Tm2 SNP2493R: 5'CTGCCAGTATATAACGGTCTACCG3'
3.2	Assay 2 to check susceptible or resistant 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: Mobaci, Monalbo, Moneymaker Homozygous resistant allele Tm2 present: Moperou Homozygous resistant allele $Tm2^2$ present: Mocimor, Momor
5.	Preparation of DNA	Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol. Pipette each DNA sample and the PCR mix (primers, dNTP's and Taq polymerase) into individual wells for assay 1 and assay 2.
6.	PCR conditions	1. Initial denaturation step at 94°C for 3 minutes 2. 2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minutes 3. 3. Final extension step of 72°C for 10 minutes Visualize PCR product on 1-2% agarose gel.
7.	Observations	
7.1	Observation scale	

Assay 1
A: Control fragment (416bp) and Tm2 fragment (255bp)
B: Control fragment (416bp) and $Tm2^2$ fragment (214bp)
C: Control fragment (416bp)



Assay 2
D: Control fragment (509bp), tm2 fragment (S-allele; 381bp) and Tm2 or $Tm2^2$ fragment (R-allele; 185bp)
E: Control fragment (509bp) and Tm2 or $Tm2^2$ fragment (R-allele; 185bp)
F: Control fragment (509bp) and tm2 fragment (S-allele; 381bp)



7.2	Validation of test	Control varieties should give the expected results.
8.	Interpretation of data in terms of UPOV characteristic states	the presence of the alleles tm2, Tm2, $Tm2^2$ lead to different interpretation for characteristics 56, 57 and 58, 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 (possibly based on another resistance gene, e.g. gene Tm1).

Test result DNA marker test	tm2/tm2	Tm2/tm2 or Tm2/Tm2	$Tm2^2$/tm2 or $Tm2^2$/$Tm2^2$ or $Tm2^2$/Tm2
		(less frequent)	(more frequent)
56 Strain 0	[1] absent	[9] resistant	[9] resistant
57 Strain 1	[1] absent	[9] resistant	[9] resistant
58 Strain 2	[1] absent	[1] absent	[9] resistant

(b) Taqman PCR

1.	Pathogen	Tomato mosaic virus

2.	Functional gene	Tm2/2^2 (with two alleles for resistance Tm2 and Tm2^2 and one allele for susceptibility tm2)
3.	Primers	TOMV RES Forward: 5'-CTCAATCATTTCCTCCAAATCTC-' TOMV RES Reverse: 5'-GGGAAATGTCTTAAGTACTGCCA-3' TOMV SUS Forward: 5'-GAAGCATTCCCTCCAAATATT-3' TOMV SUS Reverse: 5'-GGTAATGTCTTAAGCACTGCCAG-3' TOMV Probe Res TM2^2: 5'-Texas Red-CTACTTTAGTGTAGACCGT-BHQ2-3' TOMV Probe Res TM2: 5'-Atto 532-CAACTTTACGGTAGACC-BHQ1-3' TOMV Probe SUS: 5'-6FAM-TGCTTTATGGTAGACAGT-BHQ1-3' The probes are MGB probes or XS probes, designed with a temperature of 65°C.
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: Mobaci, Monalbo, Moneymaker Homozygous resistant allele Tm2 present: Moperou Homozygous resistant allele Tm2^2 present: Mocimor, Momor
5.	Preparation of DNA	Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol. Pipette each DNA sample and a commercial real-time PCR mastermix (primers, probes) into individual wells. Analyse the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction conditions suitable for the mastermix used.
6.	PCR conditions	1. Initial denaturation step at 94°C for 2-10 minutes (mastermix dependent) 2. 2. 40 cycles at 94°C for 15 sec, 60°C 1 min. Every cycle ends with plate reading
7.	Observations	
7.1	Observation scale	
7.2	Validation of test	Control varieties should give the expected results. In case of Ct/Cq 35-40: repeat the test.
8.	Interpretation of data in terms of UPOV characteristic states	the presence of the alleles tm2, Tm2, Tm2^2 lead to different interpretation for characteristics 56, 57 and 58, 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 (possibly based on another resistance gene, e.g. gene Tm1).

Probe	Ct/Cq	Interpretation
TOMV Probe Res TM2^2	<35	resistance allele Tm2^2 present
	N/A	resistance allele Tm2^2 absent
TOMV Probe Res TM2	<35	resistance allele Tm2 present
	N/A	resistance allele Tm2 absent
TOMV Probe SUS	<35	Susceptible allele tm2 present
	N/A	Susceptible allele tm2 absent

Test result DNA marker test	tm2/tm2	Tm2/tm2 or Tm2/Tm2	Tm2^2/tm2 or Tm2^2/Tm2^2 or Tm2^2/Tm2
		(less frequent)	(more frequent)
56 Strain 0	[1] absent	[9] resistant	[9] resistant
57 Strain 1	[1] absent	[9] resistant	[9] resistant
58 Strain 2	[1] absent	[1] absent	[9] resistant

This protocol was validated by a ring-test with three different laboratories (Interlaboratory Comparative Test Report, INVITE 2023). If a different protocol is used, the laboratory must validate its method in comparison to the reference method to show that the alternative protocol gives the same results. Validation of an alternative test should be carried on at least 50 varieties, with 12 individuals per variety. The varieties should cover all available outcomes as evenly as possible. After validation, the report and results should be peer-reviewed by one (preferably 2) of the EO's performing resistance testing.

Ad. 63: Resistance to *Phytophthora infestans* (Pi)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Phytophthora infestans*
3.	Host species	*Solanum lycopersicum*
5.	Isolate	highly pathogenic on tomato
6.	Establishment isolate identity	biotest
7.	Establishment pathogenicity	biotest
8.	Multiplication inoculum	
8.1	Multiplication medium	V8 Agar or PDA or Malt Agar medium
8.2	Multiplication variety	susceptible tomato variety
8.3	Plant stage at inoculation	4 weeks
8.4	Inoculation medium	water
8.5	Inoculation method	spraying
8.6	Harvest of inoculum	wash spores from wetted plates
8.7	Check of harvested inoculum	count spores
8.8	Shelflife/viability inoculum	4 h after chilling at 8-10°C
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Moneymaker, Saint-Pierre
	Resistant	Phantasia, Sixtina
9.5	Test facility	glasshouse
9.6	Temperature	18°C
9.7	Light	after inoculation darkness during 24 h, thereafter 10 h darkness per 24 h
9.9	Special measures	humidity tent during four days after inoculation
10.	Inoculation	
10.1	Preparation inoculum	wash spores from sporulating leaves, chill at 8-10°C chilling will induce zoospore release Remark: Use fresh spores from repeated infection cycles on tomato plants during 3 weeks before inoculation
10.2	Quantification inoculum	count sporangiospores; adjust to 10^4 spores per ml
10.3	Plant stage at inoculation	10 leaves developed (6 to 7 weeks)
10.4	Inoculation method	spraying
10.7	Final observations	5-7 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Symptoms: water-soaked lesions, yellowing, and death
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls heterozygous varieties may have a slightly lower level of expression of resistance
12.	Interpretation of data in terms of UPOV characteristic states	absent [1] severe symptoms present [9] no or mild symptoms
13.	Critical control points	resistance is only well-expressed in the adult plant

Ad. 64: Resistance to *Pseudopyrenochaeta lycopersici* (ex *Pyrenochaeta lycopersici*) (Pl)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Pyrenochaeta lycopersici*
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	GEVES[18] (FR)
5.	Isolate	e.g. strain Pl 21
6.	Establishment isolate identity	On susceptible plant
8.	Multiplication inoculum	
8.1	Multiplication medium	Messiaen agar or synthetic medium
8.4	Inoculation medium	Autoclaved grains (e.g. barley)
8.5	Inoculation method	Mix grains (e.g. 1 kg) with inoculum (e.g. medium from 2 Petri dishes with mycelium)
8.6	Harvest of inoculum	After 3 weeks
9.	Format of the test	
9.1	Number of plants per genotype	At least 20 plants
9.3	Control varieties	Susceptible: Marmande verte, Montfavet 63-5 Resistant: Garance and (*S. lycopersicum* x S. *habrochaites*) Emperador
9.4	Test design	Add non-inoculated plants
9.5	Test facility	Greenhouse or climatic chamber
9.6	Temperature	20°C
9.7	Light	At least 12h
10.	Inoculation	
10.1	Preparation inoculum	Homogenize the contaminated grains and mix with soil (volume ration of grains to soil ca. 1:5)
10.3	Plant stage at inoculation	3-4 leaf stage
10.4	Inoculation method	Transplanting of plantlets in a mixture of soil and contaminated grains
10.7	Final observations	40 days post inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Class 0: no necrotic lesion on roots Class 1: few small and uncoloured necrotic lesions Class 2: some brown necrotic lesions clearly visible (less than half the surface of the main root) Class 3: several brown necrotic lesions clearly visible (more than half the surface of the main root) Class 4: complete necrosis or destruction of the main root
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	Any variety judged to be of the same resistance level or higher than Garance is judged as resistant. Classes 0, 1 and 2 are commonly judged as resistant – Note 9 Classes 3 and 4 are commonly judged as susceptible – Note 1
13.	Critical control points	Pathogenicity maybe lost after 3 weeks growing on an agar medium.

Ad. 65: Resistance to *Stemphylium* spp. (Ss)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

[18] GEVES: matref@geves.fr

1.	Pathogen	*Stemphylium* spp. e.g. *Stemphylium solani* (see note below)
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	GEVES[19] (FR)
7.	Establishment pathogenicity	biotest
8.1	Multiplication medium	PDA (12 hours per day under near-ultraviolet light to induce sporulation) or V8-Agar
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Monalbo
	Resistant	Motelle, "Motelle x Monalbo" (border)
9.5	Test facility	greenhouse or climate cell
9.6	Temperature	24°C
9.7	Light	12 hours minimum
9.9	Special measures	incubation in tunnel with 100% relative humidity or humidity tent closed 5 days after inoculation, after this, 80% RH until end.
10.	Inoculation	
10.1	Preparation inoculum	sporulating plates (8.1) are scraped and air-dried overnight. The next day plates are soaked and stirred for 30 min in a beaker with demineralized water, or sporulating plates are scraped with water with Tween20. The resulting suspension is sieved through a double layer of muslin.
10.2	Quantification inoculum	$5x10^3$ to $5x10^5$ spores per ml
10.3	Plant stage at inoculation	20-22 days (three expanded leaves)
10.4	Inoculation method	spraying
10.7	Final observations	4-10 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	0. no symptoms 1. some very rare lesions plus yellowing onleaves, and no symptoms on cotyledons 2. some lesions on leaves and cotyledons 3. many lesions on leaves, and cotyledons attached 4. coalescence of lesions, and cotyledons falling 5. total drying of the first two or the first three leaves, and cotyledons fallen
11.3	Validation of test	Symptoms on Motelle x Monalbo should be a little bit stronger than on Motelle. Symptoms on Monalbo should be much stronger than on Motelle.
12.	Interpretation of data in terms of UPOV characteristic states	Resistance absent [1] strong symptoms Resistance present [9] weak symptoms or no symptoms When the resistance level is just below the lower border of resistance, the test should be repeated one or two times before a final decision is taken
13.	Critical control points	Individual isolates may differ slightly in pathogenicity. Some isolates of *Stemphylium* cannot be classified easily as either *Stemphylium solani* or a related species. These *Stemphylium* isolates may still be useful for identifying resistance to *Stemphylium solani*.

[19] GEVES, matref@geves.fr

Ad. 66: Resistance to *Pseudomonas syringae* pv. *tomato* (Pst)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Pseudomonas syringae* pv. *tomato*
2.	Quarantine status	-
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	GEVES[20] (FR)
5.	Isolate	-
7.	Establishment pathogenicity	biotest
8.	Multiplication inoculum	
8.1	Multiplication medium	e.g. King's B agar medium, darkness
8.2	Multiplication variety	susceptible variety
8.4	Inoculation medium	water
8.8	Shelflife/viability inoculum	plates become old after 10 days
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	Not applicable
9.3	Control varieties	
	Susceptible	Monalbo, Moneymaker
	Resistant	Ontario 7710, "Monalbo x Ontario 7710", Fuzzer
9.5	Test facility	greenhouse or growth chamber
9.6	Temperature	day: 22° C, night: 16° C or 20°C
9.7	Light	12 hours
9.9	Special measures	humidity tent needed for 3 days or longer
10.	Inoculation	
10.1	Preparation inoculum	wash off spores from plate and addv a drop of surfactant to the bacterial suspension. Plate should be less than 2-4 days old.
10.2	Quantification inoculum	OD 0.1 or less, supported by dilution plating. Density 10^6 colony forming units per ml
10.3	Plant stage at inoculation	three leaves expanded (20-22 days)
10.4	Inoculation method	spraying a bacterial suspension on leaves
10.7	Final observations	8 days after inoculation or longer
11.	Observations	
11.1	Method	visual
11.2	Observation scale	bacterial speck, greasy in appearance with marginal chlorosis pinpoint lesions can be observed on resistant plants < 1.0 mm
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] bacterial speck present [9] no symptoms or pinpoint lesions
13.	Critical control points	Strains may lose virulence in storage

[20] GEVES, matref@geves.fr

Ad. 67: Resistance to *Ralstonia solanacearum* - Race 1 (Rs: 1)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Ralstonia solanacearum* – Race 1
2.	Regulatory status	See EPPO Global database: https://gd.eppo.int
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	-
5.	Isolate	Race 1 (Race 1 has a wide host range, including tomato. Race 3 has a narrow host range, also including tomato.)
8.	Multiplication inoculum	
8.1	Multiplication medium	Yeast Peptone Glucose (YPG) Agar or PYDAC Special conditions: 25-30°C (Race 3 usually needs 20-23°C)
8.5	Inoculation method	2 ml of inoculum placed at the foot of each plantlet prior to transplanting
8.8	Shelf life/viability inoculum	suspension in sterile distilled water at 15°C (<1 year)
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Floradel
	Resistant	Caraïbo
9.5	Test facility	climate room
9.6	Temperature	day: 26-30°C; night: 25°C
9.7	Light	10 - 12 hours
9.9	Special measures	high humidity
10.	Inoculation	
10.2	Quantification inoculum	10^7 colony forming units per ml
10.3	Plant stage at inoculation	3 to 4 well-developed leaves (3 weeks)
10.7	Final observations	3 weeks after inoculation
11.	Observations	in intermediate resistant varieties, bacteria could be present in the lower part of the plant
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] symptoms present [9] no symptoms, or less than resistant standard

Ad. 68: Resistance to Tomato yellow leaf curl virus (TYLCV)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

(i) agroinoculation method

1.	Pathogen	Tomato yellow leaf curl virus (TYLCV)
2.	Regulatory status	See EPPO Global Database: https://gd.eppo.int
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Dr. Eduardo R. Bejarano, Plant Genetics Laboratory, HMS UMA-CSIC[21]
5.	Isolate	Alm:Pep:99, strain IL
8.	Multiplication inoculum	
8.1	Multiplication medium	YEP/Kanamycin.
8.3	Plant stage at inoculation	3-4 leaf
8.4	Inoculation medium	YEP

[21] Source of inoculum: HMS UMA (CSIC) edu_rodri@uma.es, INIA resistencias@inia.es

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)
8.8	Shelf life/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 28ºC.
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	2
9.3	Control varieties	
	Susceptible	Moneymaker, Marmande
	Resistant	Delyca, Montenegro
9.5	Test facility	Glasshouse or climatic chamber with permission to confined use of LMO/GMO
9.6	Temperature	23-25°C
9.7	Light	16 h
9.9	Special measures	The transformed *Agrobacterium tumefaciens* is a living modified organism (LMO; or genetically modified organism (GMO)) for which further regulations may apply.
10.	Inoculation	
10.1	Preparation inoculum	Streak the surface of the frozen A. tumefaciens stock tube and submerge in 5ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 28°C. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28ºC. Centrifuge the saturated culture for 20 min at 3500 rpm and discard supernatant
10.2	Quantification inoculum	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 (dpi)
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
11.4	Off-types	
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. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

(ii) White fly inoculation method

1.	Pathogen	Tomato yellow leaf curl virus (TYLCV) IL strain
2.	Quarantine status	See EPPO Global Database: https://gd.eppo.int
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Spain[22]
5.	Isolate	TYLCV-IL La Mayora
8.	Multiplication inoculum	White flies

[22] Source of inoculum; IHSM, CSIC quillamon@eelm.csic.es, INIA resistencias@inia.es

8.1	Multiplication medium	
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	Two replicates
9.3	Control varieties	
	Susceptible	Moneymaker, Marmande
	Resistant	Delyca, Montenegro
9.5	Test facility	Greenhouse/plastic tunnel
9.9	Special measures	prevent spread of white-flies
10.	Inoculation	
10.3	Plant stage at inoculation	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-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).

Ad. 69: Resistance to Tomato spotted wilt virus – Pathotype 0 (TSWV: 0)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

Resistance to strain 0 to be tested in a bio-assay (method i) or in a DNA marker test (method ii), if appropriate.

(i) bio-assay

1.	Pathogen	Tomato spotted wilt virus, Pathotype 0 (TSWV: 0)
2.	Regulatory status	See EPPO Global database: https://gd.eppo.int
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[23] (NL), GEVES[24] (FR)
5.	Isolate	pathotype 0, preferably a thrips-transmission deficient variant
6.	Establishment isolate identity	symptomatic leaves may be stored below -70°C
7.	Establishment pathogenicity	Biotest
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	Monalbo, Momor, Montfavet 63-5, Moneymaker
	Resistant	Bodar, Mospomor
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

[23] Naktuinbouw, resistentie@naktuinbouw.nl
[24] GEVES, matref@geves.fr

10.3	Plant stage at inoculation	one or two expanded leaves
10.4	Inoculation method	mechanical, rubbing with a suitable abrasive on cotyledons, inoculum suspension < 10°C
10.7	Final observations	7 -21 days after inoculation
11.	Observations	
11.1	Method	Visual, comparative
11.2	Observation scale	Symptoms: top mosaic, bronzing, various malformations, strong necrosis can be a sign of hypersensitivity
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] symptoms present [9] no symptoms or symptoms of hypersensitivity
13.	Critical control points	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.

(iii) DNA marker test

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

<table>
<tr><td>1.</td><td>Pathogen</td><td>Tomato spotted wilt virus – pathotype 0</td></tr>
<tr><td>2.</td><td>Functional gene</td><td>Sw-5b</td></tr>
<tr><td>3.</td><td>Primers</td><td></td></tr>
<tr><td>3.1</td><td>Susceptible alleles</td><td>Sw5-Vat1-F: 5’-ACAACATCAAACAATGTTAGCC-3’
Sw5-Vat2-F: 5’-CATCAAACAATGCAGTTAGCC-3’</td></tr>
<tr><td>3.2</td><td>Resistant allele</td><td>Sw5-Res-F: 5’-ATCAACCAATACAGCCTAACC-3</td></tr>
<tr><td>3.3</td><td>Universal reverse</td><td>Sw5-universal-R: 5’-TTTCTCCCTGCAAGTTCACC-3’</td></tr>
<tr><td>3.3</td><td>Allele specific probes</td><td>Sw5-Sus1: 5’-VIC-TACATTATGAAGGGTTAACAAG-MGB-NFQ-3’
Sw5-Sus2: 5’-6FAM-ACAACAGAGGGTTAACAAGTTTAGG-BHQ1-3’
Sw5-Res: 5’-TEXAS RED-TGGGCGAAAATCCCAACAAG-BHQ2-3’</td></tr>
<tr><td>4.</td><td>Format of the test</td><td></td></tr>
<tr><td>4.1</td><td>Number of plants per genotype</td><td>at least 20 plants</td></tr>
<tr><td>4.2</td><td>Control varieties</td><td>homozygous susceptible allele 1 present:
Moneymaker
homozygous susceptible allele 2 present:
Mountain Magic
homozygous resistant allele present:
Montealto
Heterozygous 1 (allele for resistance and allele 1 for susceptibility present): Bodar
Heterozygous 2 (allele for resistance and allele 2 for susceptibility present): Sharmita</td></tr>
<tr><td>5.</td><td>Preparation of DNA</td><td>Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol. Pipette each DNA sample and a commercial real-time PCR mastermix into individual wells. Analyse the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction conditions suitable for the mastermix used.</td></tr>
<tr><td>6.</td><td>PCR conditions</td><td>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.</td></tr>
<tr><td>7.</td><td>Observations</td><td></td></tr>
<tr><td>7.1</td><td>Observation scale</td><td>
<table>
<tr><th>probe</th><th>Ct/Cq</th><th>interpretation</th></tr>
<tr><td rowspan="2">Sw5-Sus1</td><td><35</td><td>susceptible allele sw5b-1 present</td></tr>
<tr><td>N/A</td><td>susceptible allele sw5b-1 absent</td></tr>
<tr><td rowspan="2">Sw5-Sus2</td><td><35</td><td>susceptible allele sw5b-2 present</td></tr>
<tr><td>N/A</td><td>susceptible allele sw5b-2 absent</td></tr>
<tr><td rowspan="2">Sw5-Res</td><td><35</td><td>resistance allele Sw-5b present</td></tr>
<tr><td>N/A</td><td>resistance allele Sw-5b absent</td></tr>
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7.2	Validation of the test	Control varieties should give the expected results. In case of Ct/Cq 35-40: repeat the test.
8.	Interpretation of data in terms of UPOV characteristic states	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).

This protocol was validated by a ring-test with three different laboratories (Interlaboratory Comparative Test Report, INVITE 2023). If a different protocol is used, the laboratory must validate its method in comparison to the reference method to show that the alternative protocol gives the same results. Validation of an alternative test should be carried on at least 50 varieties, with 12 individuals per variety. The varieties should cover all available outcomes as evenly as possible. After validation, the report and results should be peer-reviewed by one (preferably 2) of the EO's performing resistance testing.

Ad. 70: Resistance to *Leveillula taurica* (Lt)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Leveillula taurica*
2.	Quarantine status	-
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	no long term storage method is available
8.1	Multiplication medium	detached leaves of a susceptible host plant
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Monalbo, Montfavet 63-5
	Resistant	Radiance
10.	Inoculation	
10.3	Plant stage at inoculation	adult plants
10.4	Inoculation method	natural infection, mainly by wind dispersal of spores
10.7	Final observations	before maturity of fruits
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Symptoms: Yellow chlorotic spots on upper side of leaves, mycelium on abaxial side of leaves
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] symptoms present [9] no symptoms, or same level as the resistant control.
13.	Critical control points	Check cleistothecia under microscope to confirm presence of *Leveillula* and not another powdery mildew. Plant stage dependent action of resistance can cause difficulties in the interpretation

Ad. 71: Resistance to *Pseudoidium neolycopersici* (ex *Oidium neolycopersici*) (Pn (ex On))

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	*Oidium neolycopersici*
2.	Quarantine status	-
3.	Host species	*Solanum lycopersicum*
5.	Isolate	see remark under 13
7.	Establishment pathogenicity	biotest
8.	Multiplication inoculum	

8.1	Multiplication medium	plant
8.3	Plant stage at inoculation	24°C during the day; 18°C during the night
8.4	Inoculation medium	water
8.5	Inoculation method	see 10.4
8.6	Harvest of inoculum	by washing off
8.7	Check of harvested inoculum	check for contaminants under microscope
8.8	Shelf life/viability inoculum	1-2 hours
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	Not applicable
9.3	Control varieties	
	Susceptible	Momor, Montfavet 63-5
	Resistant	Romiro, PI 247087
9.5	Test facility	glasshouse
9.6	Temperature	20°C or 18/24°C
9.7	Light	12 hours
10.	Inoculation	
10.1	Preparation inoculum	collect spores in water
10.2	Quantification inoculum	10^4 conidia/ml
10.3	Plant stage at inoculation	3 weeks
10.4	Inoculation method	by spraying on leaves or dredging of leaves
10.7	Final observations	7-18 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	0. no sporulation 1. necrotic points and sometimes locally restricted sporulation 2. moderate sporulation 3. abundant sporulation
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] Moderate or abundant sporulation present [9] No or restricted sporulation
13.	Critical control points	Resistance-breaking isolates should be avoided. Resistance to *O. neolycopersici* is usually race-specific. However, as long as a differential series of tomato genotypes with well-defined resistances is lacking, it will remain hard to conclude that different races of *O. neolycopersici* exist.

Ad. 72: Resistance to Tomato torrado virus (ToTv)

Observations should be made in at least one growing cycle. One growing cycle may be enough if the results are sufficient for taking a decision regarding distinctness, uniformity and stability, and for the description of the characteristic.

1.	Pathogen	Tomato torrado virus
2.	Quarantine status	in regions with temperate climate
3.	Host species	*Solanum lycopersicum*
7.	Establishment pathogenicity	biotest
8.	Multiplication inoculum	
8.1	Multiplication medium	*Nicotiana tabacum* 'Xanthi'
8.3	Plant stage at inoculation	cotyledon to first leaf
8.5	Inoculation method	see 10.4
8.6	Harvest of inoculum	after 3 weeks
8.7	Check of harvested inoculum	plants yellow, systemic infection
8.8	Shelf life/viability inoculum	instable at room temperature
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.3	Control varieties	
	Susceptible	Daniela
	Resistant	Matias
9.5	Test facility	glasshouse

9.6	Temperature	23°C during the day; 21°C during the night
9.7	Light	16 hours
10.	Inoculation	
10.3	Plant stage at inoculation	14 days
10.4	Inoculation method	with ice-cold 0,01 M PBS pH 7 and carborundum
10.5	First observation	7 days after inoculation
10.6	Second observation	14 days after inoculation
10.7	Final observations	18 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	necrotic spots on the top leaves
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] necrotic spots present present [9] No symptoms
13.	Critical control points	ToTV is transmitted by white fly (*Bemisia tabaci*). Produce inoculum with ice-cold mortar and pestle. During inoculation the temperature should be below 25°C.

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO/TQ-044/5-Rev – *Solanum lycopersicum* L. - tomato

Link to e-TQ:

https://online.plantvarieties.eu/backOfficeFormQuestions?viewFormId=18337&viewFormType=TQ&viewFormLang=EN&speciesIds=LYC01&status=1,2