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 14/04/2021

Entry into force on 01/06/2021

TABLE OF CONTENTS

CPVO-TP/044/4-Rev.5

1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of ***Solanum lycopersicum* L.** (including rootstocks), as well as to rootstocks belonging to *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. and *Solanum lycopersicum* L. x *Solanum cheesmaniae* (L. Riley)Fosberg.

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 (UPOV Document TG/1/3 http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (http://www.upov.int/tgp/en/) and the relevant UPOV Test Guideline TG/44/11 Rev. 3 dated 29/10/2019 (https://www.upov.int/edocs/tgdocs/en/tg044.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.06.2021**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

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.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report.

If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

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 http://cpvo.europa.eu/applications-and-examinations/technical-examinations/submission-of-plant-material-s2-publication in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.
- the plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

2.3 Informing about problems on the submission of material

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

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

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

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.

The optimum stage of development for the assessment of each characteristic is indicated by a number in the third column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.

3.4 Test design

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

3.4.2 When resistance characteristics are used for assessing distinctness, uniformity and stability, records must be taken under conditions of controlled infection and, unless otherwise specified, on at least 20 plants.

3.4.3 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 Special tests for additional characteritics

In accordance with Article 23 of Implementing Rules N° 874/2009 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection

(a) Seed propagated agricultural and vegetable species and fruit species specified on the annex 1 of the entrustment requirements

The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

(b) Vegetatively propagated agricultural and vegetable species

The variety collection shall comprise variety descriptions; no living reference collection is required. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material

(a) Seed propagated agricultural and vegetable species and fruit species specified on the annex 1 of the entrustment requirements

The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

(b) Ornamental species, vegetatively propagated agricultural and vegetable species and fruit species not specified on the annex 1 of the entrustment requirements

The EO shall obtain living plant material of reference varieties as and when those varieties need to be included in growing trials or other tests.

3.6.3 Range of the variety collection

The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO.

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection

The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue.

3.6.5 Maintenance and renewal/update of a living variety collection

(a) Seed propagated species

The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

(b) Vegetatively propagated species

The EO shall maintain the variety collection under appropriate growing conditions (e.g. glasshouse, orchard, in vitro), where it shall be ensured that the plants are adequately irrigated, fertilised, pruned and protected from harmful pests and diseases. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material or by checking the identity of the new material against the variety description.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

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) and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

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. color 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. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

4.2.1 For the assessment of uniformity, 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 variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 **Technical Protocols covering both seed-propagated and vegetatively propagated varieties**
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 of blade (characteristic 10)
c) Peduncle: abscission layer (characteristic 19)
d) Fruit: green shoulder (before maturity) (characteristic 21)
e) Fruit: green stripes (before maturity) (characteristic 25)
f) Fruit: size (characteristic 26)
g) Fruit: shape in longitudinal section (characteristic 28)
h) Fruit: number of locules (characteristic 36)
i) Fruit: colour (at maturity) (characteristic 37)
j) Resistance to *Meloidogyne incognita* (Mi) (characteristic 43)
k) Resistance to *Verticilium* sp. (Va and Vd) – Race 0 (characteristic 44)
l) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 0EU/1US (characteristic 45.1)
m) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 1EU/2US (characteristic 45.2)
n) Resistance to *Tomato mosaic virus* (ToMV) – Strain 0 (characteristic 48.1)
o) Resistance to *Tomato spotted wilt virus* (TSWV) – Strain 0 (characteristic 55)

5.4 If other characteristics than those from the Technical Protocol are used for the selection of varieties to be included into the growing trial, the EO 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 tests 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 or by specific legislation on plant health. In the latter case, the CPVO should be informed.

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.

Asterisked characteritics
In the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

6.2. States of expression and corresponding notes

In the case of qualitative and pseudo-qualitative characteristics, all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	Note
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9

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
(*)	Asterisked 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.**	**VG**	**<u>Seed-propagated varieties only</u>: Seedling: anthocyanin coloration of hypocotyl**		
QL			absent	Colt, Heinz 8104, Mogeor, Momorvert, VTM215	1
			present	Montfavet H 63.4, DG-039	9
2. (+)	**2. (*)**	**VG**	**Plant: growth type**		
QL			determinate	Campbell 1327, Prisca	1
G			indeterminate	Marmande VR, Saint-Pierre, San Marzano 2	2
3.	**3.**	**VG/MS**	**<u>Only varieties with plant growth type determinate</u>: Plant: number of inflorescences on main stem (side shoots to be removed)**		
QN			few	Campbell 1327	3
			medium	Montfavet H 63.4	5
			many	Prisca	7
4. (+)	**4.**	**VG**	**Stem: anthocyanin coloration**		
QN			absent or very weak	Mogeor, Momorvert	1
			weak	Montfavet H 63.5	3
			medium	Rondello	5
			strong	Grinta, Nemato	7
			very strong		9
5. (+)	**5.**	**VG/MS**	**<u>Only varieties with plant growth type indeterminate</u>: Stem: length of internode**		
QN		**(a)**	short	Dombito, Manific, Paso, Trend	3
			medium	Montfavet H 63.5	5
			long	Berdy, Calimero	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6. (+)	**6.**	**VG/MS**	**<u>Only varieties with plant growth type indeterminate:</u> Plant: height**		
QN			very short	Cherry Belle	1
			short	Carson, Despina	3
			medium	Brooklyn, Buffalo, Vision	5
			long	Classy, Clarence, Climberly, Massada	7
			very long	Daydream, Minired	9
7. (+)	**7. (*)**	**VG**	**Leaf: attitude**		
QN		**(a)**	erect		1
			semi-erect	Allround, Drakar, Vitador	3
			horizontal	Aromata, Triton	5
			semi-drooping	Montfavet H 63.5	7
			drooping	Multolino, Naram, Tibet	9
8.	**8.**	**VG/MS**	**Leaf: length**		
QN		**(a)**	short	Nelson, Red Robin, Tiny Tim	3
			medium	Lorena	5
			long	Montfavet H 63.5	7
9.	**9.**	**VG/MS**	**Leaf: width**		
QN		**(a)**	narrow	Marmande VR, Red Robin, Tiny Tim	3
			medium		5
			broad	Saint-Pierre	7
10. (+)	**10. (*)**	**VG**	**Leaf: type of blade**		
QL		**(a)**	pinnate	Mikado, Pilot, Red Jacket	1
G			bipinnate	Lukullus, Saint-Pierre	2

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
11. (+)	**11.**	**VG**	**Leaf: size of leaflets**		
QN		**(a)**	very small	Minitom	1
			small	Tiny Tim	3
			medium	Marmande VR, Royesta	5
			large	Daniela, Hynema	7
			very large	Dombo	9
12.	**12.**	**VG**	**Leaf: intensity of green colour**		
QN		**(a)**	light	Macero II, Poncette, Rossol	3
			medium	Lucy	5
			dark	Allround, Daniela, Lorena, Red Robin	7
13. (+)	**13.**	**VG**	**Leaf: glossiness**		
QN		**(a)**	weak	Daniela	3
			medium	Marmande VR	5
			strong	Guindilla	7
14. (+)	**14.**	**VG**	**Leaf: blistering**		
QN		**(a)**	weak	Daniela	3
			medium	Marmande VR	5
			strong	Delfine, Tiny Tim	7
15. (+)	**15.**	**VG**	**Leaf: attitude of petiole of leaflet in relation to main axis**		
QN		**(a)**	semi-erect	Blizzard, Marmande VR	3
			horizontal	Sonatine	5
			semi-drooping	Montfavet H63.5	7
16. (+)	**16.**	**VG/MS**	**Inflorescence: type**		
QN			mainly uniparous	Dynamo	1
			equally uniparous and multiparous	Harzfeuer	2
			mainly multiparous	Marmande VR	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
17.	**17. (*)**	**VG**	**Flower: colour**		
QL			yellow	Exota, Marmande VR	1
			orange	Orama, Pericherry	2
18. (+)	**18.**	**VG**	**Flower: pubescence of style**		
QL			absent	Campbell 1327	1
			present	Saint-Pierre	9
19. (+)	**19. (*)**	**VG**	**Peduncle: abscission layer**		
QL			absent	Aledo, Bandera, Count, Lerica	1
G			present	Montfavet H 63.5, Roma	9
20. (+)	**20. (*)**	**VG/MS**	**Only for varieties with peduncle abscission layer present: Peduncle: length**		
QN			short	Cerise, Ferline, Montfavet H 63.18, Rossol	3
			medium	Dario, Primosol	5
			long	Erlidor, Ramy, Ranco	7
21. (+)	**21. (*)**	**VG**	**Fruit: green shoulder (before maturity)**		
QL		**(b)**	absent	Felicia, Rio Grande, Trust	1
G			present	Daniela, Montfavet H 63.5	9
22. (+)	**22.**	**VG**	**Only for varieties with a green shoulder: Fruit: extent of green shoulder (before maturity)**		
QN		**(b)**	very small	Daniela	1
			small	Ballet, Cristy, Firestone, Siluet	3
			medium	Erlidor, Foxy, Montfavet H 63.5	5
			large	Cobra, Delisa, Epona, Manific	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
23. (+)	**23.**	**VG**	**<u>Only for varieties with a green shoulder:</u> Fruit: intensity of green colour of shoulder (before maturity)**		
QN		**(b)**	light	Ballet, Daniela, Juboline	3
			medium	Montfavet H 63.5, Siluet	5
			dark	Ayala, Erlidor, Xenon	7
24. (+)	**24.**	**VG**	**Fruit: intensity of green colour excluding shoulder (before maturity)**		
QN		**(b)**	very light	Clarée	1
			light	Capello, Daniela, Duranto, Durinta, Trust	3
			medium	Marmande, Rody	5
			dark	Ayala, Centella, Tatiana, Uragano	7
			very dark	Verdi	9
25. (+)	**25 (*).**	**VG**	**Fruit: green stripes (before maturity)**		
QL		**(b)**	absent	Daniela	1
G			present	Green Zebra, Tigerella	9
26.	**26. (*)**	**VG**	**Fruit: size**		
QN		**(c)**	very small	Cerise, Sweet 100	1
			small	Early Mech, Europeel, Roma	3
			medium	Alphamech, Diego	5
			large	Carmello, Ringo	7
G			very large	Erlidor, Lydia, Muril	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
27.	**27. (*)**	**VG/MS**	**Fruit: ratio length/diameter**		
QN		**(c)**	very small (very compressed)	Campbell 28, Marmande VR	1
			small (moderately compressed)	Alicia	3
			medium	Early Mech, Peto Gro	5
			large (moderately elongated)	Rimone, Rio Grande	7
			very large (very elongated)	Elko, Macero II	9
28. (+)	**28. (*)**	**VG**	**Fruit: shape in longitudinal section**		
PQ		**(c)**	flattened	Campbell 28, Marmande VR	1
			oblate	Montfavet H 63.4, Montfavet H 63.5	2
			circular	Cerise, Moneymaker	3
			oblong	Early Mech, Peto Gro	4
			cylindric	Hypeel 244, Macero II, San Marzano 2	5
			elliptic	Alcaria, Castone	6
			cordate	Valenciano	7
			ovate	Dualrow, Soto	8
			obovate	Duquesa, Estelle, Rimone, Rio Grande	9
			pyriform	Europeel	10
G			obcordate	Cuero de Ponente, Magno	11
29. (+)	**29. (*)**	**VG**	**Fruit: ribbing at peduncle end**		
QN		**(c)**	absent or very weak	Calimero, Cerise	1
			weak	Early Mech, Hypeel 244, Melody, Peto Gro, Rio Grande	3
			medium	Montfavet H 63.4, Montfavet H 63.5	5
			strong	Campbell 1327, Carmello, Count	7
			very strong	Costoluto Fiorentino, Ingrid, Marmande VR	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
30. (+)	**30.**	**VG**	**Fruit: depression at peduncle end**		
QN		**(c)**	absent or very weak	Europeel, Heinz 1706, Rossol, Sweet Baby	1
			weak	Futuria, Melody	3
			medium	Carmello, Count, Fandango, Saint-Pierre	5
			strong	Ballon Rouge, Marmande VR	7
31. (+)	**31.**	**VG/MS**	**Fruit: size of peduncle scar**		
QN		**(c)**	very small	Cerise, Heinz 1706, Sweet Baby	1
			small	Early Mech, Peto Gro, Rio Grande	3
			medium	Montfavet H 63 4, Montfavet H 63 5	5
			large	Apla, Campbell 1327, Carmello, Fandango, Flora Dade	7
			very large	Marmande VR	9
32. (+)	**32.**	**VG/MS**	**Fruit: size of blossom scar**		
QN		**(c)**	very small	Cerise, Early Mech, Europeel, Heinz 1706, Peto Gro, Rio Grande	1
			small	Montfavet H 63.4, Montfavet H 63.5	3
			medium	Alphamech, Apla, Carmello, Floradade	5
			large	Campbell 1327, Count, Marmande VR, Saint-Pierre	7
			very large	Rozova Magia	9
33. (+)	**33.**	**VG**	**Fruit: shape at blossom end**		
QN		**(c)**	indented	Marmande VR, Super Mech	1
			indented to flat		2
			flat	Montfavet H 63.4, Montfavet H 63.5	3
			flat to pointed	Cal J, Early Mech, Peto Gro	4
			pointed	Europeel, Heinz 1706, Hypeel 244, Roma VF	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
34. (+)	**34.**	**VG/MS**	**Fruit: diameter of core in cross section in relation to total diameter**		
QN		**(c)**	very small	Cerise	1
			small	Early Mech, Europeel, Heinz 1706, Peto Gro, Rio Grande, Rossol	3
			medium	Montfavet H 63.4, Monfavet H 63.5	5
			large	Apla, Campbell 1327, Carmello, Count, Fandango, Floradade	7
			very large	Marmande VR, Valenciano	9
35. (+)	**35.**	**VG**	**Fruit: thickness of pericarp**		
QN		**(c)**	very thin	Cerise	1
			thin	Marmande VR	3
			medium	Carmello, Europeel, Floradade, Heinz 1706, Montfavet H 63.5	5
			thick	Cal J, Daniela, Ferline, Peto Gro, Rio Grande	7
			very thick	Myriade, Rondex	9
36. (+)	**36. (*)**	**VG/MS**	**Fruit: number of locules**		
QN		**(c)**	only two	Early Mech, Europeel, San Marzano	1
			two and three	Alphamech, Futuria	2
			three and four	Montfavet H 63.5	3
			four, five or six	Raïssa, Tradiro	4
G			more than six	Marmande VR	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
37. (+)	**37. (*)**	**VG**	**Fruit: colour at maturity**		
PQ		**(c)**	cream	Jazon, White Mirabell	1
			yellow	Goldene Königin, Yellow Pear	2
			orange	Sungold	3
			pink	Aichi First	4
			red	Daniela, Ferline, Montfavet H 63.5	5
			brown	Ozyrys	6
G			green	Green Grape, Green Zebra	7
38. (+)	**38. (*)**	**VG**	**Fruit: colour of flesh (at maturity)**		
PQ		**(c)**	cream	Jazon	1
			yellow	Jubilée	2
			orange	Sungold	3
			pink	Regina	4
			red	Ferline, Saint-Pierre	5
			brown	Ozyrys	6
			green	Green Grape, Green Zebra	7
39.	**39.**	**VG**	**Fruit: glossiness of skin**		
QN		**(c)**	weak	Josefina	1
			medium	Roncardo	2
			strong	Mecano	3
40. (+)	**41. (*)**	**VG**	**Fruit: firmness**		
QN		**(c)**	very soft	Marmande VR	1
			soft	Trend	3
			medium	Cristina	5
			firm	Fernova, Konsul, Tradiro	7
			very firm	Daniela, Karat, Lolek	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
41. (+)	**43.**	**MS**	**Time of flowering**		
QN			early	Feria, Primabel	3
			medium	Montfavet H 63.5, Prisca	5
			late	Manific, Saint-Pierre	7
42. (+)	**44. (*)**	**MG**	**Time of maturity**		
QN			very early	Dolcevita, Sungold, Sweet Baby	1
			early	Bianca, Rossol, Shiren	3
			medium	Gourmet, UC 82B	5
			late	Arletta, Durinta	7
			very late	Daniela	9
43. (*)	**46. (*)**	**VG**	**Resistance to *Meloidogyne incognita* (Mi)**		
(+)			susceptible	Casaque Rouge	1
QN			moderately resistant	Campeon, Tyonic	2
G			highly resistant	Anahu x Casaque Rouge	3
44. (*)	**47. (*)**	**VG**	**Resistance to *Verticillium* sp. (Va and Vd) - Race 0**		
(+)					
QL			absent	Anabel, Marmande verte	1
G			present	Dianela, Marmande VR	9
45. (+)	**48.**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
45.1 (*)	**48.1 (*)**		- **Race 0EU/1US**		
QL			absent	Marmande, Marmande verte, Resal	1
G			present	Gourmet, Larissa, Marporum, "Marporum x Marmande verte", Mohawk, Motelle, Riesling	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
45.2 (*)	**48.2 (*)**	**VG**	- **Race 1EU/2US**		
QL			absent	Cherry Belle, Marmande verte, Marporum, Ranco, Roma	1
G			present	Agostino, "Motelle x Marmande verte", Odisea, Tradiro	9
45.3	**48.3**	**VG**	- **Race 2EU/3US**		
QL			absent	Marmande verte, Marporum, Motelle	1
			present	Alliance, Florida, Ivanhoe, "Marmande verte x Florida", Murdoch, Tributes	9
46. (+)	**49.**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**		
QL			absent	Motelle	1
			present	Momor	9
47. (+)	**50.**	**VG**	**Resistance to *Passalora fulva* (Pf) (ex *Fulvia fulva* (Ff))**		
47.1	**50.1**		- **Race 0**		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine, Sonato, Vemone	9
47.2	**50.2**	**VG**	**- Group A**		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine, Sonato	9
47.3	**50.3**	**VG**	**- Group B**		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine, Sonato, Vemone	9
47.4	**50.4**	**VG**	**- Group C**		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
47.5	**50.5**	**VG**	**- Group D**		
QL			absent	Monalbo	1
			present	Estrella, Sonatine, Vemone	9
47.6	**50.6**	**VG**	**- Group E**		
QL			absent	Monalbo	1
			present	Sonatine	9
48. (+)	**51.**		**Resistance to *Tomato mosaic virus* (ToMV)**		
48.1 (*)	**51.1**	**VG**	**- Strain 0**		
QL			absent	Monalbo, Moneymaker	1
G			present	Mobaci, Mocimor, Momor, Moperou	9
48.2	**51.2**	**VG**	**- Strain 1**		
QL			absent	Monalbo, Moneymaker	1
			present	Mocimor, Momor, Moperou	9
48.3	**51.3**	**VG**	**- Strain 2**		
QL			absent	Monalbo, Moneymaker, Moperou	1
			present	Mobaci, Mocimor, Momor	9
49. (+)	**52.**	**VG**	**Resistance to *Phytophthora infestans* (Pi)**		
			absent	Heinz 1706, Saint Pierre	1
QL			present	Fline, Heline, Pieraline, Pyros	9
50. (+)	**53.**	**VG**	**Resistance to *Pyrenochaeta lycopersici* (Pl)**		
QL			absent	Marmande verte	1
			present	Garance	9
51. (+)	**54.**	**VG**	**Resistance to *Stemphylium* spp. (Ss)**		
QL			absent	Monalbo	1
			present	Motelle	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
52. (+)	**55.**	**VG**	**Resistance to *Pseudomonas syringae* pv. tomato (Pst)**		
QL			absent	Monalbo	1
			present	Ontario 7710	9
53. (+)	**56.**	**VG**	**Resistance to *Ralstonia solanacearum* (Rs) - Race 1**		
			absent	Floradel	1
QL			present	Caraïbo	9
54. (+)	**57.**	**VG**	**Resistance to *Tomato yellow leaf curl virus* (TYLCV)**		
			absent	Moneymaker, Marmande	1
QL			present	Anastasia, Mohawk, TY 20	9
55. (+)	**58.**	**VG**	**Resistance to *Tomato spotted wilt virus* (TSWV) – Strain 0**		
QL			absent	Moneymaker, Montfavet H 63.5, Mountain Magic	1
G			present	Bodar, Montealto	9
56. (+)	**59.**	**VG**	**Resistance to *Leveillula taurica* (Lt)**		
QL			absent	Montfavet H 63.5	1
			present	Atlanta	9
57. (+)	**60.**	**VG**	**Resistance to *Oidium neolycopersici* (On) (ex *Oidium lycopersicum* (Ol))**		
			absent	Montfavet H 63.5	1
QL			present	Romiro	9
58. (+)	**61.**	**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 on the plant, stem and leaf should be done after a fruit set on at least five trusses and before ripening of the second truss. In the case of determinate varieties, all observations on the plant and leaves should be done after a fruit set on the second truss. Observations should be done before deterioration of the leaves.

(b) Observations should be made on the plant before maturity.

(c) Observations should be made on fruits at maturity 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

1
absent

9
present

Ad. 2: Plant: growth type

Determinate (1): This type produces a limited number of trusses. The number of trusses is different among varieties (Note: can be influence by agro climatic conditions). In this type, the number of leaves or internodes between inflorescence is irregular within a plant and varies from one to three. The stem ends with an inflorescence and no lateral shoots are produced.

This type includes some so-called "semi-determinate" varieties which do not have consistently three leaves or internodes between inflorescences, and show semi-determinate growth, for example, with the termination of the stem with the 9th inflorescence (e.g. 'Prisca' type) or at higher than 20th inflorescence (e.g. Early Pack type).

Indeterminate (2): In this type, as a rule, three leaves or internodes are observed between inflorescences. After every group of three leaves, the plant produces three buds: the terminal bud is transformed into an inflorescence and one of the two lateral buds starts the prolongation of stem. Plants of this type grow with the continuous repeat of this growth pattern.

It should be noted that only two leaves or internodes might be observed between inflorescences in some parts of plants in a certain group of indeterminate variety types (e.g. varieties originating from 'Daniela'). These varieties nevertheless are indeterminate.

This type includes 'Marmande' and 'Costoluto Fiorentino' types which might be considered to be categorised into an intermediate class between indeterminate and determinate, but they always have three leaves or internodes between inflorescences. They should therefore be categorised into the indeterminate type.

Ad. 4. Stem: anthocyanin coloration of upper third

Most of the varieties are classed 1 to 5. Expression of anthocyanin is influenced by day temperature. Under greenhouse conditions, the variation is rather low.

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

The length of the internode should be observed/measured 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 1st and 4th trusses. In case of measurements, this 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

The height of the plant should be measured at one time for the whole trial, e.g. 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 in respect to the main stem should be observed.

3 semi-erect | 5 horizontal | 7 semi-drooping | 9 drooping

Ad. 10: Leaf: type of blade

Pinnate leaf: primary leaflets do not bear secondary leaflets
Bipinnate leaf: primary leaflets again are pinnate, so they bear secondary leaflets

1 pinnate | 2 bipinnate

Ad. 11: Leaf: size of leaflets (in middle of leaf)

The size of leaflet should be observed in the middle of the leaf.

Ad. 13: Leaf: glossiness

The glossiness of the leaf should be observed in the middle of the plant.

Ad. 14: Leaf: blistering

Caution is required to avoid 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. The blistering should be observed in the middle third of the plant.

blistering

creasing

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

3
semi-erect

5
horizontal

7
semi-drooping

The attitude should be observed in the middle third of the plant.

Ad. 16: Inflorescence: type

The number of uniparous and multiparous trusses on the second and third truss of 10 plants should be counted. When the ratio of uniparous to multiparous is 40-60 per cent, the expression of the characteristic should correspond to note "2".

uniparous multiparous (biparous)

multiparous (triparous)

Ad. 18: Flower: pubescence of style

Some varieties with pubescence of style "present" may have only rare and small hairs at the base of the style.

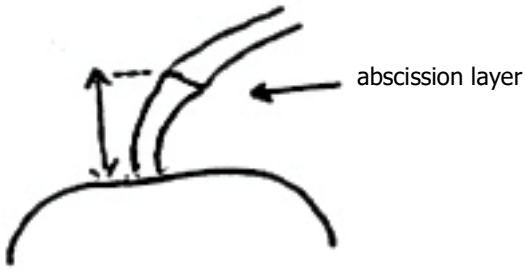
Ad. 19: Peduncle abscission layer

1
absent

9
present

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: Only varieties with peduncle abscission layer present: Peduncle: length



Ad. 21: Fruit: green shoulder (before maturity)

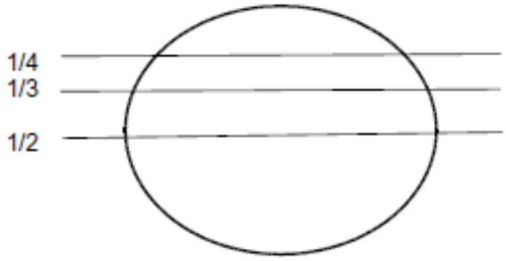
The gene for green shoulder might not be clearly expressed in some conditions, which is why it is important to have the example variety 'Daniela' to observe the expression of these characteristics.

1
absent

9
present

Ad. 22: Fruit: extent of green shoulder (before maturity)

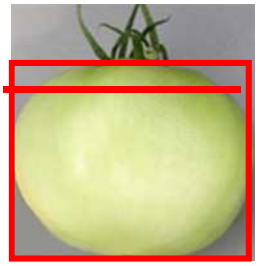
The gene for green shoulder might not be clearly expressed in some conditions, which is why it is important to have the example variety 'Daniela' to observe the expression of these characteristics.



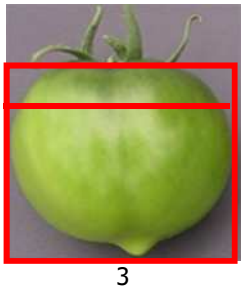
3: small (1/4)

5: medium (1/3)

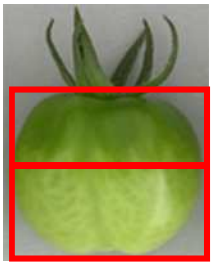
7: large (1/2)



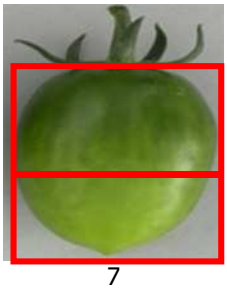
1
very small



3
small



5
medium



7
large

Ad. 24: Fruit: intensity of green colour excluding shoulder (before maturity)

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. The gene for green shoulder might not be clearly expressed in some conditions, which is why it is important to have the example variety 'Daniela' to observe the expression of these characteristics.

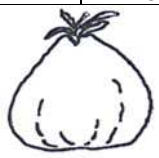
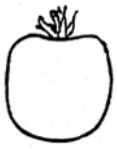
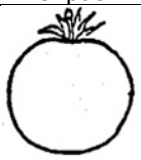
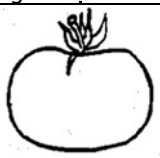
Ad. 25: Fruit: green stripes (before maturity)

The green stripes should be observed before maturity, *excluding* the green shoulder.

1
absent

9
present

Ad. 28: Fruit: shape in longitudinal section

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

The apex is considered to be the part that is farthest from the peduncle end.

Ad. 29: Fruit: ribbing at peduncle end

For ease of observation, it is recommended to remove the peduncle.


1
absent or very weak


3
weak


5
medium


7
strong


9
very strong

Ad. 30: Fruit: depression at peduncle end

1
absent or very weak

3
weak

5
medium

7
strong

Ad. 31: Fruit: size of peduncle scar

The size of the peduncle scar has to be observed as an absolute characteristic, i.e. irrespective of the size of the fruit. The peduncle should be removed and the green ring observed (not the full scar).

Ad. 32: Fruit: size of blossom scar

The size of the blossom scar has to be observed as an absolute characteristic, i.e. irrespective of the size of the fruit.

Ad. 33: Fruit: shape at blossom end

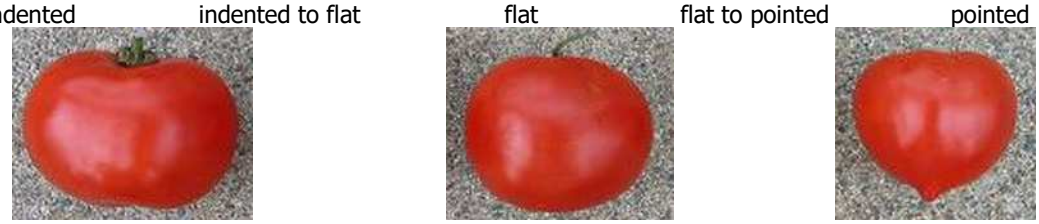
1
indented

2
indented to flat

3
flat

4
flat to pointed

5
pointed

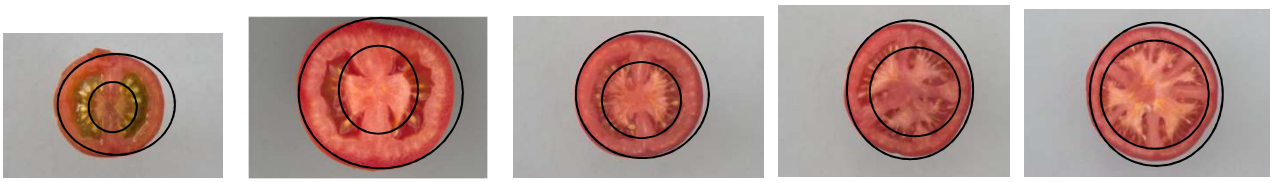


1
indented

3
flat

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

The thickness of the pericarp has to be observed as an absolute characteristic, i.e. irrespective of the size of the fruit.

Ad. 36: Fruit: number of locules

This characteristic is assessed by making cross sections of representative shaped and sized fruits but excluding the first and last fruits from the truss.

1	2	3	4	5
only two	two and three	three and four	four, five or six	more than six

Ad. 37: Fruit: colour (at maturity)

The colour at maturity has to be observed after a full change of colour, when placenta is found clearly in the cross section.

It should be noted that parent lines homozygous for the RIN gene do not ripen at all. In that case this characteristic is not applicable.

Ad. 38: Fruit: colour of flesh (at maturity)

The colour at maturity has to be observed at maturity.

Ad. 40: Fruit: firmness

Method

Harvesting stage:..... fruits should be harvested when they are completely coloured.

Determining firmness:..... determine by hand the firmness of the fruits compared to the standard varieties.

Ad. 41: Time of flowering

For staked varieties, this characteristic is assessed by observing the flowering date of the third flower on the second [and third trusses], plant by plant. It is recommended not to record the time of flowering on the first truss, as the expression on the first truss is more influenced by the seed vigour and the plantation quality.

The date of flowering is recorded by the plot average, truss by truss.

For determinate non-staked varieties, it is recommended to grow them on pruned stakes on the main stem and to record the characteristics in the same way as those for 'staked varieties'. On non-staked crops, this characteristic cannot be observed easily due to the branching of the plant.

Ad. 42: Time of maturity

This characteristic is assessed by observing the date of maturity of the first fully ripe fruit on the second truss, plant by plant. It is recommended not to record the time of maturity on the first truss, as the expression on the first truss is more influenced by the seed vigour and the plantation quality.

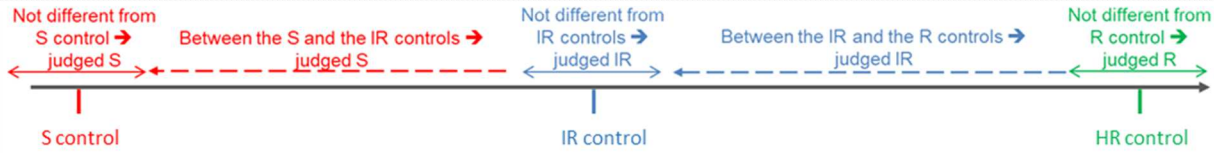
The date of maturity is recorded by the plot average, truss by truss.

Ad. 43: Resistance to *Meloidogyne incognita* (Mi)

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	preferably resistant to powdery mildew
8.3	Plant stage at inoculation	2 leaf stage
8.5	Inoculation method	deposit of piece of contaminated roots in soil (around 5-10g per 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	30 plants, plus at least 10 non-inoculated plants to observe if a possible lack of germination is due to nematode or not
9.2	Number of replicates	-
9.3	Control varieties	Susceptible: Casaque Rouge Intermediate resistant: Campeon and Tyonic Resistant: Anahu x Casaque Rouge
9.4	Test design	3 repetitions of 10 plants in different trays by variety to allow statistical analysis
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 comportment of controls but should not be above 26°C
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 must have an equal repartition on the soil.
10.3	Plant stage at inoculation	seed
10.4	Inoculation method	plants sown in soil contaminated with infested root homogeneously mixed with soil
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

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

11.3	Validation of test	Validation on controls. Expected comportment of controls: Susceptible: most plants at classes 3 and 4, at most 2 plants can be observed at class 2. Resistant: most plants at classes 0 and 1, at most 2 plants can be observed at class 2. Intermediate resistant: clearly different from other controls with majority of plants around class 2.
11.4	Off-types	resistant varieties may have a few plants with a few galls
12.	Interpretation of data in terms of UPOV characteristic states	Variety very similar to resistant control is judged as resistant. Variety very similar to susceptible control is judged as susceptible. Variety very similar to intermediate resistant control is judged as intermediate resistant. If significantly different from resistant and intermediate resistant control (notations are between resistant and intermediate resistant controls), the variety is judged as intermediate resistant. If significantly different from intermediate resistant and susceptible control (notations are between intermediate resistant and susceptible controls), the variety is judged as susceptible. If results are not clear, statistical analysis is advised.
		
13.	Critical control points	Avoid rotting of roots; high temperature causes breakdown of resistance. In case of aggressive test, put seeds in a layer of non-contaminated soil or decrease the quantity of inocula.

Ad. 44: Resistance to *Verticillium* sp. (Va and Vd)

1. Pathogen.....	*Verticillium dahliae* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[4] (NL) and GEVES[5] (*F*)
5. Isolate	Race 0 (e.g. strain Toreilles 4-1-4-1)
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 106 per ml
8.8 Shelf life/viability inocula.....	1 day at 4°C
9. Format of the test	
9.1 Number of plants per genotype	35 seed for 24 plants
9.2 Number of replicates.....	Not applicable
9.3 Control varieties	
Susceptible	Flix, Marmande verte, Clarion, Santonio, Anabel
Resistant.....	Monalbo, Elias, Monalbo x Marmande verte, Daniela, Marmande VR
9.4 Test design	20 plants inoculated, 2 blanks at least
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 inocula.....	aerated, liquid culture (8.4)
10.2 Quantification inocula	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 Final observations.....	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 then 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.

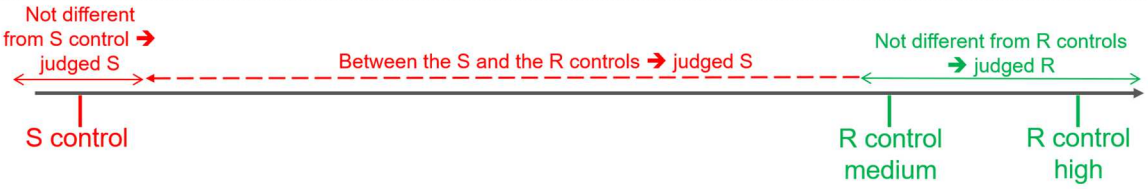
[4] Naktuinbouw; resistentie@naktuinbouw.nl
[5] GEVES; matref@geves.fr

Ad. 45.1 + 45.2 + 45.3: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) - Race 0EU/1US, Race 1EU/2US and Race 2EU/3US

1.	Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
2.	Quarantine status	-
3.	Host species	*Solanum lycopersicum* L.
4.	Source of inoculum	GEVES[6] (FR), INIA[7] (ES) or Naktuinbouw[8] (NL)
5.	Isolate	Race 0EU/1US (e.g. strains Orange 71 or PRI 20698 or Fol 071), race 1EU/2US (e.g. strains 4152, PRI40698 or RAF 70) and race 2EU/3US The test protocol has been validated in a CPVO co-funded project[9] with these isolates/pathotypes. Individual strains may vary in pathogenicity
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 Resistant: Marporum, Larissa, "Marporum x Marmande verte", Motelle, Gourmet, Mohawk; 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 Resistant: Tradiro, Odisea,"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, Ivanhoe, Tributes, Murdoch, "Marmande verte x Florida"
9.4	Test design	-
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
11.	Observations	
11.1	Method	visual
11.2	Observation scale	

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

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 comportment of controls: Susceptible: most plants in 2 and 3, at most 10% of the plants can be observed at classes 0 and 1 Resistant: most plants in 0 and 1, at most 10% of the plants can be observed at classes 2 and 3
12.	Interpretation of data in terms of UPOV characteristic states	If not different from either one resistant level controls, the variety is judged resistant If lower level than the medium resistant level control, the variety is judged susceptible. If no clear results, statistics must be used.
		
13.	Critical control points	-

Ad. 46: Resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl)

1. Pathogen.....	*Fusarium oxysporum* f. sp. *radicis-lycopersici*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[10] (NL) and GEVES[11] (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 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
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 blanks
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
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; frequent renewal of races may be needed because of loss of pathogenicity

[10] Naktuinbouw; resistentie@naktuinbouw.nl
[11] GEVES; matref@geves.fr

Ad. 47.1 - 47.7: Resistance to *Passalora fulva* (Pf) (ex *Fulvia fulva* (Ff))

1. Pathogen.....	*Passalora fulva* (ex *Fulvia fulva)*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[12] (NL) or GEVES[13] (FR)
5. Isolate	Race group 0, A, B, C, D, and E
6. Establishment isolate identity	with genetically defined differentials from GEVES (FR) A breaks Cf-2, B Cf-4, C Cf-2&4, D Cf-5, E Cf-2&4&5
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 Shelf life/viability inoculum.....	4 hours, keep cool
9. Format of the test	
9.1 Number of plants per genotype	more than 20
9.2 Number of replicates.....	Not applicable
9.3 Control varieties	
Susceptible:	Monalbo, Moneymaker
Resistant for race 0:	Angela, Estrella, Sonatine, Sonato, Vemone, Vagabond, IVT 1149, Vagabond × IVT 1149, IVT 1154
Resistant for race group A:.....	Angela, Estrella, Sonatine, Sonato
Resistant for race group B:.....	Angela, Estrella, Sonatine, Sonato, Vemone
Resistant for race group C:.....	Angela, Estrella, Sonatine
Resistant for race group D:.....	Estrella, Sonatine, Vemone
Resistant for race group E:.....	Sonatine, Jadviga, Rhianna, IVT 1154
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
9.9 Special measures	depending on facility and weather, there may be a need to raise the humidity, e.g. humidity tent closed 3-4 days after inoculation and after this, 66% until 80% closed during 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
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
11.4 Off-types	excessively high humidity may cause rugged brown spots on all leaves

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. Store good culture at -80°C.
For practical purposes, it is not possible to keep plants longer than 14 days inside a tent.

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

Ad.48.1 – 48.3: Resistance to *Tomato mosaic virus* (ToMV) - Strains 0, 1 and 2

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).

(i) Bio-assay

1. Pathogen.....	*Tomato mosaic virus*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[14] (NL) or GEVES[15] (FR) or INIA[16] (ES, strain 0)
5. Isolate	Strain 0_T (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 ($Tm2^2$)
7. Establishment pathogenicity.....	on susceptible plant
8. Multiplication inoculum	
8.1 Multiplication medium.....	living plant
8.2 Multiplication variety.....	e.g. Moneymaker, Marmande
8.7 Check of harvested inoculum....	option: on *Nicotiana tabacum* "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum.....	fresh>1 day, desiccated>1year
9. Format of the test	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates.....	1 replicate
9.3 Control varieties	
Susceptible	Marmande, Monalbo
Resistant for ToMV: 0 and 2	Mobaci
Resistant for ToMV: 0 and 1	Moperou
Resistant with necrosis.....	"Monalbo x Momor"
Resistant.....	Gourmet
9.4 Test design.....	blank treatment with PBS and carborundum or similar buffer
9.5 Test facility	Glasshouse or climate room
9.6 Temperature.....	24 to 26°C
9.7 Light	12 hours or longer
9.8 Season	symptoms are more pronounced in summer
10. Inoculation	
10.1 Preparation inoculum	1 g leaf with symptoms with 10 ml PBS or similar buffer Homogenize, add carborundum to buffer (1 g/30 ml)
10.6 Plant stage at inoculation	cotyledons or 2 leaves
10.4 Inoculation method.....	gentle rubbing
10.7 Final observations.....	11-21 days after inoculation
11. Observations	
11.1 Method.....	visual
11.2 Observation scale	Symptoms of susceptibility: Mosaic in top, leaf malformation Symptoms of resistance (based on hypersensitivity): Local Necrosis, Top necrosis, Systemic Necrosis
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls

Remark: in some heterozygous varieties a variable proportion of plants may have severe systemic necrosis or some necrotic spots while the other plants have no symptoms. This proportion may vary between experiments.

12. Interpretation of 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.

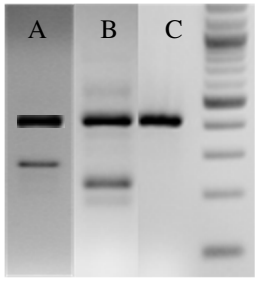
Remark: Strain INRA Avignon 6-5-1-1 is recommended for ToMV: 0. This strain causes a striking yellow Aucuba mosaic.

[14] Naktuinbouw; resistentie@naktuinbouw.nl
[15] GEVES; matref@geves.fr
[16] INIA; resistencias@inia.es

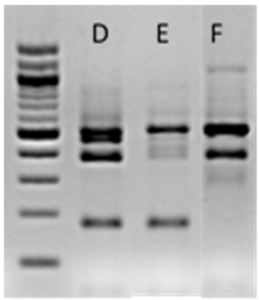
(ii) DNA marker test

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). Specific aspects:

1. Pathogen ..... *Tomato mosaic virus*
2. Functional gene ..... Tm2/2^2 (with two alleles for resistance Tm2 and Tm22 and one allele for susceptibility tm2)
3. Primers
3.1. Assay 1 to check resistance
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
resistance allele .....Outer primer TM2-748F:5'CGGTCTGGGGAAAACAACTCT3'
.....Outer primer TM2-1256R:5'CTAGCGGTATACCTCCACATCTCC3'
.....TM2-SNP901misR: 5'GCAGGTTGTCCTCCAAATTTTCCATC3'
.....TM2-SNP901misF: 5'CAAATTGGACTGACGGAACAGAAAGTT3'
4. Format of the test
4.1 Number of plants per genotype .....at least 20 plants
4.2 Control varieties.....homozygous susceptible allele tm2 present:
(*Solanum lycopersicum*) Mobaci, Monalbo, Moneymaker
Homozygous resistant allele Tm2 present: (*Solanum lycopersicum*): Moperou
Homozygous resistant allele Tm2^2 present: (*Solanum lycopersicum*): 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 into individual wells for assay 1 and for assay 2. 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 3 minutes
2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minutes
3. Final extension step of 72°C for 10 minutes
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 Tm22 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)

8. Interpretation of test results ..... the presence of the alleles tm2, Tm2, Tm2^2 lead to different interpretation for characteristics 48.1, 48.2 and 48.3, see table. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene Tm1).

Test result DNA marker test	tm2/tm2	Tm2/tm2 or Tm2/Tm2	$Tm2^2$/tm2 or $Tm2^2$/$Tm2^2$ or $Tm2^2$/Tm2
		(occurs incidentally)	
48.1 Strain 0	[1] absent	[9] resistant	[9] resistant
48.2 Strain 1	[1] absent	[9] resistant	[9] resistant
48.3 Strain 2	[1] absent	[1] absent	[9] resistant

Ad. 49: Resistance to *Phytophthora infestans* (Pi)

1. Pathogen..... *Phytophthora infestans*
3. Host species ..... *Solanum lycopersicum*
4. Source of inoculum ..... -
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 sporangiospores
8.8 Shelf life/viability inoculum..... 4 h after chilling at 8-10°C
9. Format of the test
9.1 Number of plants per genotype 20
9.2 Number of replicates..... Not applicable
9.3 Control varieties
Susceptible ..... Saint Pierre, Heinz 1706
Resistant..... Pieraline, Heline, Pyros, "Pieraline x Pieralbo", Fline
Remark:..... heterozygote varieties may have a slightly lower level of expression of resistance.
9.5 Test facility..... glasshouse
9.6 Temperature..... 18°C
9.7 Light ..... after inoculation darkness during 24 hours, thereafter 10 hour darkness per 24 hours
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 104 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
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. 50: Resistance to *Pyrenochaeta lycopersici* (Pl)

1.	Pathogen	*Pyrenochaeta lycopersici*
2.	Quarantine status	No
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	GEVES[17] (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 of contaminated 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
9.2	Number of replicates	1 replicate
9.3	Control varieties	Susceptible: Marmande verte 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
10.3	Plant stage at inoculation	3-4 leaf stage
10.4	Inoculation method	Transplanting of plantlets in a mixture of soil (e.g. 3750 ml of soil with 750 ml of inoculum)
10.7	Final observations	40 days post inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Class 0: no necrosic 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 pivot) Class 3: several brown necrotic lesions clearly visible (more than half the surface of the pivot) Class 4: complete necrosis or destruction of the pivot
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

[17] GEVES: matref@geves.fr

Ad. 51: Resistance to *Stemphylium* spp. (Ss)

1. Pathogen.....	*Stemphylium* spp. e.g. *Stemphylium solani* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	GEVES[18] (FR)
5. Isolate	-
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
8.1 Multiplication medium.....	PDA (12 hours per day under near-ultraviolet light to induce sporulation) or V8
9. Format of the test	
9.1 Number of plants per genotype	20 at least
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible:	Monalbo
Resistant:	Motelle, F1 Motelle x Monalbo
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% 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 Twee. The spore suspension is sieved through a double layer of muslin.

10.2 Quantification inoculum.....	$5.10^3 – 10^5$ 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	Symptoms: necrotic lesions on cotyledons and leaves; yellowing 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 (11.2)
present	[9]	no symptoms, or less than resistant standard

13. Critical control points:.....	8.1 and 10.1

Note: 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*.

[18] GEVES: matref@geves.fr

Ad. 52: Resistance to *Pseudomonas syringae* pv. *tomato* (Pst)

1. Pathogen.....	*Pseudomonas syringae* pv. *tomato* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	GEVES[19] (FR) or Naktuinbouw[20] (NL)
5. Isolate	-
6. Establishment isolate identity	-
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
8.1 Multiplication medium.....	King's B agar medium, darkness
8.2 Multiplication variety.....	Susceptible variety
8.4 Inoculation medium.....	water
8.8 Shelf life/viability inoculum.....	plates become old after 10 days
9. Format of the test	
9.1 Number of plants per genotype	20 at least
9.2 Number of replicates.....	Not applicable
9.3 Control varieties	
Susceptible:	Monalbo
Resistant:	Ontario 7710, "Monalbo x Ontario 7710", Tradiro, Hypeel 45
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. Plate should be less than 2-4 days old.
10.2 Quantification inoculum.....	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 < 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

[19] GEVES: matref@geves.fr
[20] Naktuinbouw:c resistentie@naktuinbouw.nl

Ad. 53: Resistance to *Ralstonia solanacearum* (ex. *Pseudomonas solanacearum)* (Rs) - Race 1

1. Pathogen.....	*Ralstonia solanacearum* (ex *Pseudomonas solanacearum)*
2. Quarantine status	yes (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	-
5. Isolate	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	20
9.2 Number of replicates.....	Not applicable
9.3 Control varieties	
Susceptible:	Floradel
Resistant:	Caraibo
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.....	density 10^7 colony forming units per ml
10.3 Plant stage at inoculation	three to four well-developed leaves (3 weeks)
10.4 Inoculation method	
10.7 Final observations.....	3 weeks after inoculation
11. Observations.....	In intermediate resistance 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

Ralstonia solanacearum has a quarantine status in some countries and is on the EPPO alert list.

Ad. 54: Resistance to *Tomato yellow leaf curl virus* (TYLCV)

(i) agroinoculation method

1. Pathogen.....	*Tomato yellow leaf curl virus* (TYLCV) IL strain. (see note below)
2. Quarantine status	yes
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
6. Establishment isolate identity	
7. Establishment pathogenicity	
8. Multiplication inoculum	
8.1 Multiplication medium.....	YEP/Kanamycin.
8.2 Multiplication variety.....	
8.3 Plant stage at inoculation.....	3-4 leaf
8.4 Inoculation medium.....	YEP
8.5 Inoculation method	Stem puncture agroinfiltration. Plant agroinoculation is carried out using Agrobacterium tumefaciens transformed with plasmids containing the infectious clones (Morilla, et al. 2005. Phytopathology 95: 1089-1097)
8.6 Harvest of inoculum	
8.7 Check of harvested inoculum....	
8.8 Shelfife/viability inocula	*A. tumefaciens* stocks are maintained frozen at -80ºC in 15-20% glicerol for long term storage. Cultures to be stored are typically started from a single colony and grown in 5 ml YEP +2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC.
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	2
9.3 Control varieties	
Susceptible:	Moneymaker, Marmande
Resistant:	Delyca, Montenegro, Anastasia, TY20, Mohawk
9.4 Test design.....	
9.5 Test facility.....	Glasshouse or climatic chamber with permission to confined use of use of LMO/GMO, confinement level 1 (N-1)[22]
9.6 Temperature.....	23-25°C
9.7 Light	16 h
9.8 Season	
9.9 Special measures	Permission to confined use of OGM, at least level 1 (N-1)
10. Inoculation	
10.1 Preparation inocula.....	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 inocula	Dissolve in sterile deionize water to a final OD_{600} of 1.
10.3 Plant stage at inoculation	3-4th leaf
10.4 Inoculation method.....	Take up into a 1 ml syringe with a 27-gauge needle and few drops (about 20 µl of the culture) were deposited on 10-15 puncture wounds made with the needle into the stem of test tomato plants. Maintain on ice while inoculating plants.
10.5 First observation.....	20 days post inoculation
10.6 Second observation	30 dpi
*10.7 Final observations.....	45 dpi
11. Observations	
11.1 Method.....	visual
11.2 Observation scale	Symptoms: leaf yellowing and curling
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls

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

[22] The transformed Agrobacterium tumefaciens is a living modified organism (LMO; or genetically modified organism (GMO)) and in many countries it requires to comply with Cartagena Protocol on Biosafety in case of transboundary movement, transit, handling and use that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	severe symptoms
present	[9]	no symptoms

13. Critical control points:

TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate.

TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2.

TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

(ii) White fly inoculation method

1. Pathogen.....	*Tomato yellow leaf curl virus* (TYLCV) IL strain
2. Quarantine status	yes
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Spain[23]
5. Isolate	TYLCV-IL La Mayora
8. Multiplication inoculum	White flies
8.6 Harvest of inoculum	
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates.....	Two replicates
9.3 Control varieties	
Susceptible:	Moneymaker, Marmande,
Resistant:	Delyca, Montenegro, Anastasia, TY20, Mohawk
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).

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

Ad 55: Resistance to *Tomato spotted wilt virus* (TSWV) – Strain 0

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

(i) bio-assay

1. Pathogen.....	*Tomato spotted wilt virus* (see note below)
2. Quarantine status	yes (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[24] (NL), GEVES[25] (FR)
5. Isolate	strain 0, preferably a thrips-transmission deficient variant
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
6 Harvest of inoculum	symptomatic leaves may be stored at -70°C
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	1 replicate
9.3 Control varieties	
Susceptible	Monalbo, Momor, Montfavet H 63.5
Resistant.....	Tsunami, Bodar, Mospomor, Lisboa
9.5 Test facility	glasshouse or climatic chamber
9.6 Temperature.....	20°C
9.7 Light	12 hours or longer
9.9 Special measures	prevent or combat thrips
10. Inoculation	
10.1 Preparation inoculum	press symptomatic leaves in ice-cold buffer 0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite or similar buffer Option: sieve the leaf sap through double muslin
10.3 Plant stage at inoculation	one or two expanded leaves
10.4 Inoculation method	mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10 C
10.5 Final observations.....	7--21 days after inoculation
11. Observations	
11.1 Method.....	visual
11.2 Observation scale	Symptoms: top mosaic, bronzing, various malformations, necrosis
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	symptoms
present	[9]	no symptoms

13. Critical control points:

TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabac*i and Western flower thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties carrying the resistance gene Sw-5. TSW resistance based on Sw-5 may be detected without using the pathogen.

[24] Naktuinbouw; resistentie@naktuinbouw.nl
[25] GEVES; matref@geves.fr

(ii) DNA marker test

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

1. Pathogen ..... *Tomato spotted wilt virus*
2. Functional gene Sw-5b
3. Primers
3.1 Susceptible alleles ..... Sw5-Vat1-F: 5'-ACAACATCAAACAATGTTAGCC-3'
Sw5-Vat2-F: 5'-CATCAAACAATGCAGTTAGCC-3'
3.2 Resistant allele..... Sw5-Res-F: 5'-ATCAACCAATACAGCCTAACC-3
3.3 Universal reverse..... Sw5-universal-R: 5'-TTTCTCCCTGCAAGTTCACC-3'
3.3 Allele specific probes ..... Sw5-Sus1: 5'-VIC-TACATTATGAAGGGTTAACAAG-MGB-NFQ-3'
Sw5-Sus2: 5'-6FAM-ACAACAGAGGGTTAACAAGTTTAGG-BHQ1-3'
Sw5-Res: 5'-TEXAS RED-TGGGCGAAAATCCCAACAAG-BHQ2-3'4. Format of the test
4.1 Number of plants per genotype at least 20 plants
4.2 Control varieties..... homozygous susceptible allele 1 present:
Solanum lycopersicum) Moneymaker
homozygous susceptible allele 2 present:
Solanum lycopersicum) Mountain Magic
homozygous resistant allele present:
(Solanum lycopersicum) Montealto
heterozygous (allele for resistance and allele 1 for susceptibility present): Bodar
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 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 10 min 95 °C
2. 40 cycles 15 sec 95 °C and 1 min 60°C. Every cycle ends with a plate reading.
7. Observations
7.1 Observation scale

probe	Ct/Cq	interpretation
Sw5-Sus1	<35	susceptible allele sw5b-1 present
	N/A	susceptible allele sw5b-1 absent
Sw5-Sus2	<35	susceptible allele sw5b-2 present
	N/A	susceptible allele sw5b-2 absent
Sw5-Res	<35	resistance allele Sw-5b present
	N/A	resistance allele Sw-5b absent

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 test results .....
absent [1] susceptible allele(s) present and resistant allele absent
present [9] resistant allele present (homozygous or heterozygous)

In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism).

Ad. 56: Resistance to *Leveillula taurica* (Lt)

1. Pathogen.....	*Leveillula taurica*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	no long term storage method is available
5. Isolate	
8.1 Multiplication medium.....	detached leaves of a susceptible host plant
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible	Monalbo , Montfavet H 63.5
Resistant.....	Atlanta
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 harvest
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

Remark: Check cleistothecia under microscope to confirm presence of *Leveillula* and not another powdery mildew.

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. 57: Resistance to *Oidium neolycopersici* (On) (ex *Oidium lycopersicum)* (Ol)

1. Pathogen.....	*Oidium neolycopersici* (Powdery mildew)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	-
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	20
9.2 Number of replicates	Not applicable
9.3 Control varieties.....	
Susceptible:	Momor, Montfavet H 63.5
Resistant tomato:	Atlanta, 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. 58: Resistance to *Tomato torrado virus* (ToTv)

1. Pathogen..... *Tomato Torrado Virus*
2. Quarantine status ..... in regions with temperate climate
3. Host species ..... *Solanum lycopersicum*
4. Source of inoculum ..... -
5. Isolate
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 20
9.2 Number of replicates ..... Not applicable
9.3 Control varieties
Susceptible ..... Daniela
Resistant tomato ..... 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.

Note: Patents pending on part of the method: WO2006/085749 and WO2008/150158 and equivalents. Use solely for DUS purposes and for the development of variety descriptions by UPOV and authorities of UPOV members, courtesy to De Ruiter Seeds R&D B.V./Monsanto Invest N.V.

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/044/4-Rev.5 – *Solanum lycopersicum* L. - tomato