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

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Solanum habrochaites S. Knapp & D.M. Spooner;
Solanum lycopersicum L. x *Solanum habrochaites* S. Knapp & D.M. Spooner;
Solanum lycopersicum L. x *Solanum peruvianum* (L.) Mill.;
Solanum pimpinellifolium L. x *Solanum habrochaites* S. Knapp & D.M. Spooner

TOMATO ROOTSTOCKS

UPOV Code: SOLAN_HAB; SOLAN_LHA; SOLAN_LPE; SOLAN_PHA

Adopted on 29/02/2024

Entry into force on 01/04/2024

CPVO-TP/294/1-Rev.6 based on the version 5 of the CPVO-TP/template

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CPVO-TP/294/1-Rev.6

1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of *Solanum habrochaites* S. Knapp & D.M. Spooner; *Solanum lycopersicum* L. x *Solanum habrochaites* S. Knapp & D.M. Spooner, *Solanum lycopersicum* L. x *Solanum peruvianum* L. (Mill.) and *Solanum pimpinellifolium* L. x *Solanum habrochaites* S. Knapp & D.M. Spooner. Such varieties are generally used as rootstocks for tomato varieties (varieties of *Solanum lycopersicum* L. (*Lycopersicum esculentum* L. (Mill.)).

Rootstocks belonging to *Solanum lycopersicum* L. (*Lycopersicum esculentum* Mill.), to *Solanum lycopersicum* L. x *Solanum cheesmaniae* (L. Ridley) Fosberg or to *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. (*Lycopersicum esculentum* Mill. x *Lycopersicum pimpinellifolium* Mill.) should be covered by the most recent version of the CPVO protocol for tomato CPVO-TP/044/4-Rev.5.

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/294/1 Corr. Rev. 4 dated 25/10/2022 (https://www.upov.int/edocs/tgdocs/en/tg294.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.04.2024**. 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 growing cycle.

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

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 four weeks after the date of the request for technical examination by the CPVO and in any case preferably before the submission period of the plant material.

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

In cases where the Examination Office identifies issues during the course of the technical examination that may lead to a negative report, the Examination Office shall inform the CPVO and in urgent cases the applicant/holder as soon as such issues become obvious.

1.3.3 Sample keeping in case of problems

As far as feasible the Examination Office shall keep a representative sample of any relevant testing material of the candidate variety and reference variety(ies) if the technical examination has resulted in a negative report. As soon as possible, the CPVO shall inform the Examination Office when the material can be destroyed.

2. MATERIAL REQUIRED

2.1 Plant material requirements

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

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

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

The testing of a variety may be concluded when the entrusted examination office can determine with certainty the outcome of the test.

3.2 Testing Place

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

3.3 Conditions for Conducting the Examination

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

3.4 Test design

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

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

3.5 Special tests for additional characteristics

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 characteristics 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
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.

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

3.6.3 Range of the variety collection
The living variety collection shall cover at least those common knowledge varieties that are suitable to grow in the 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 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.

The inventory shall take into account the list of varieties which are the subject of an on-going application for protection or official registration (candidate varieties).

3.6.5 Maintenance and renewal/update of a living variety collection
The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required.
Living material in variety collections representing varieties for which a DUS test was carried out at that EO shall be renewed after verification in a side-by-side comparison. In case where no living material is available anymore in the collection, such verification could be done with any other test that has proven to give similar results between the material in the collection and the new material.

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.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

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.

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

4.1.5 Method of observation

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

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

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

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

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

4.2 Uniformity

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

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

4.2.3 For the assessment of uniformity, 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 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) Fruit: green shoulder (characteristic 11)

b) Fruit: shape in longitudinal section (characteristic 17)

c) Fruit: colour at maturity (characteristic 19)

d) Autonecrosis (characteristic 21)

e) Resistance to *Meloidogyne incognita* (characteristic 22)

f) Resistance to *Verticillium* sp. (Va and Vd) – Race 0 (characteristic 23)

g) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 0EU/1US (characteristic 24.1)

h) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 1EU/2US (characteristic 24.2)

i) Resistance to *Fusarium oxysporum* f. sp*. lycopersici* – Race 2EU/3US (characteristic 24.3)

5.4 If characteristics other than those mentioned in the list of grouping characteristics and/or from the table of characteristics and/or from the Technical Questionnaire – sections 5 and 7 are used for the selection of varieties to be included into the growing trial, the 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 characteristics
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

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

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

6.3 Example Varieties

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

6.4 Legend

For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2
(*)	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**	**Seedling: anthocyanin coloration of hypocotyl**		
QL			absent		1
			present	Beaufort	9
2. (+)	**2.**	**VG**	**Plant: height**		
QN			very short		1
			very short to short		2
			short	Radar	3
			short to medium		4
			medium	Maxifort	5
			medium to tall		6
			tall	Beaufort	7
			tall to very tall		8
			very tall		9
3.	**3.**	**VG**	**Stem: anthocyanin coloration of upper third**		
QN		**(a)**	absent or very weak		1
			very weak to weak		2
			weak	Arnold	3
			weak to medium		4
			medium	Beaufort	5
			medium to strong		6
			strong	Montezuma	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
4. (+)	**4.**	**VG/MS**	**Stem: length of internode**		
QN		**(a)**	very short		1
			very short to short		2
			short	Big Force	3
			short to medium		4
			medium	Maxifort	5
			medium to long		6
			long	Beaufort	7
			long to very long		8
			very long		9
5.	**5. (*)**	**VG/MS**	**Leaf: length**		
QN		**(a)**	very short		1
			very short to short		2
			short		3
			short to medium		4
			medium	Body	5
			medium to long		6
			long	Maxifort	7
			long to very long		8
			very long		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	**6. (*)**	**VG/MS**	**Leaf: width**		
QN		**(a)**	very narrow		1
			very narrow to narrow		2
			narrow		3
			narrow to medium		4
			medium	Body	5
			medium to broad		6
			broad	Emperador	7
			broad to very broad		8
			very broad		9
7. (+)	**7.**	**VG**	**Leaf: size of leaflets**		
QN		**(a)**	very small		1
			very small to small		2
			small	Titron	3
			small to medium		4
			medium	Big Force	5
			medium to large		6
			large	Beaufort	7
			large to very large		8
			very large	Hires 1210	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
8.	**8. (*)**	**VG**	**Leaf: intensity of green colour**		
QN		**(a)**	very light		1
			very light to light		2
			light		3
			light to medium		4
			medium		5
			medium to dark		6
			dark	Maxifort	7
			dark to very dark		8
			very dark		9
9. (+)	**9.**	**VG**	**Leaf: glossiness**		
QN		**(a)**	weak	Montezuma	1
			medium	Titron	2
			strong	Maxifort	3
10. (+)	**10.**	**VG**	**Leaf: blistering**		
QN		**(a)**	weak	Montezuma	1
			medium	Emperador	2
			strong	Body	3
11.	**11. (*)**	**VG**	**Fruit: green shoulder**		
QL		**(c)**	absent		1
G			present	Big Force, Maxifort	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12. (+)	**12. (*)**	**VG**	**Fruit: extent of green shoulder**		
QN		**(c)**	very small		1
			very small to small		2
			small	Big Force	3
			small to medium		4
			medium		5
			medium to large		6
			large	Maxifort	7
			large to very large		8
			very large		9
13.	**13. (*)**	**VG**	**Fruit: intensity of green colour of shoulder**		
QN		**(c)**	very light		1
			very light to light		2
			light		3
			light to medium		4
			medium		5
			medium to dark		6
			dark	He-man	7
			dark to very dark		8
			very dark		9
14. (+)	**14.**	**VG**	**Fruit: conspicuousness of meridian stripes**		
QN		**(c)**	very weak	He Wolf	1
			weak	Popeye	2
			medium	Body	3
			strong	Vigomax	4
			very strong		5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
15. (+)	**15.**	**VG/MS**	**Pedicel: length**		
QN		**(b)**	very short		1
			very short to short		2
			short	Titron	3
			short to medium		4
			medium	Multifort	5
			medium to long		6
			long	Beaufort	7
			long to very long		8
			very long		9
16. (+)	**16. (*)**	**VG**	**Fruit: size**		
QN		**(b)**	not developed or very small	RT303	1
			very small to small		2
			small	Body, Optifort	3
			small to medium		4
			medium	Emperador	5
			medium to large		6
			large	Titron	7
			large to very large		8
			very large		9
17. (+)	**17. (*)**	**VG**	**Fruit: shape in longitudinal section**		
PQ		**(b)**	broad oblate	He-Wolf	1
			narrow oblate	Gladiator	2
			circular	Maxifort	3
G			obovate	Forzapro	4

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
18.	**18. (*)**	**VG/MS**	**Fruit: number of locules**		
QN		**(b)**	only two	Maxifort	1
			two and three		2
19.	**19. (*)**	**VG**	**Fruit: colour at maturity**		
PQ		**(b)**	green	Big Force	1
			yellowish	Vigomax	2
			orangish	Titron	3
			reddish	Brigeor	4
20.	**20.**	**MG**	**Time of flowering**		
QN			very early		1
			very early to early		2
			early	He-Man	3
			early to medium		4
			medium	Body	5
			medium to late		6
			late	Popeye	7
			late to very late		8
			very late		9
21. (+)	**21. (*)**	**VG**	**Autonecrosis**		
QL			absent	Maxifort	1
G			present	Body	9
22. (*)	**22. (*)**	**VG**	**Resistance to *Meloidogyne incognita* (Mi)**		
(+)			susceptible	Bruce	1
QN			moderately resistant		2
G			highly resistant	Emperador	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
23. (+) (*)	**23.**	**VG**	**Resistance to *Verticillium* sp. (Va and Vd) - Race 0**		
QL			absent		1
G			present	Bruce, Emperador, King Kong	9
24. (+)	**24.**		**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
24.1 (*)	**24.1**	**VG**	**Race 0EU/1US**		
QL			absent		1
G			present	Emperador	9
24.2 (*)	**24.2**	**VG**	**Race 1EU/2US**		
QL			absent		1
G			present	Emperador	9
24.3 (*)	**24.3 (*)**	**VG**	**Race 2EU/3US**		
QL			absent	Emperador	1
G			present	Colosus	9
25. (*)	**25. (*)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**		
(+)			absent	Kemerit	1
QL			present	Emperador	9
26. (+)	**26.**		**Resistance to *Passalora fulva* (Pf)**		
26.1	**26.1**	**VG**	**Race 0**		
QL			absent	King Kong	1
			present	Bruce	9
26.2	**26.2**	**VG**	**Race A**		
QL			absent	King Kong	1
			present	Vitalfort	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
26.3	**26.3**	**VG**	**Race B**		
QL			absent	King Kong	1
			present	Bruce	9
26.4	**26.4**	**VG**	**Race C**		
QL			absent		1
			present	Vitalfort	9
26.5	**26.5**	**VG**	**Race D**		
QL			absent	King Kong	1
			present	Bruce	9
26.6	**26.6**	**VG**	**Race E**		
QL			absent	Bruce, King Kong	1
			present	Vitalfort	9
27. (+)	**27.**		**Resistance to *Tomato mosaic virus* (ToMV)**		
27.1	**27.1**	**VG**	**Strain 0**		
QL			absent		1
			present	Emperador	9
27.2	**27.2**	**VG**	**Strain 1**		
QL			absent		1
			present	Emperador	9
27.3	**27.3**	**VG**	**Strain 2**		
QL			absent		1
			present	Emperador	9
28 (+)	**28.**	**VG**	**Resistance to *Pyrenochaeta lycopersici* (Pl)**		
QL			absent		1
			present	Emperador	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
29. (+)	**29.**	**VG**	**Resistance to *Stemphylium* spp. (Ss)**		
QL			absent		1
			present	Body	9
30. (+)	**30.**	**VG**	**Resistance to *Tomato yellow leaf curl virus* (TYLCV)**		
QL			absent		1
			present		9
31. (+)	**31.**	**VG**	**Resistance to *Tomato spotted wilt virus* (TSWV)**		
QL			absent	Emperador	1
			present	Enpower	9
32. (+)	**32.**	**VG**	**Resistance to *Oidium neolycopersici* (On)**		
QL			absent		1
			present	Multifort	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) Observations on the plant, stem and leaves should be done after a fruit set on at least five trusses and before ripening of the second truss. Observations should be done before deterioration of the leaves.

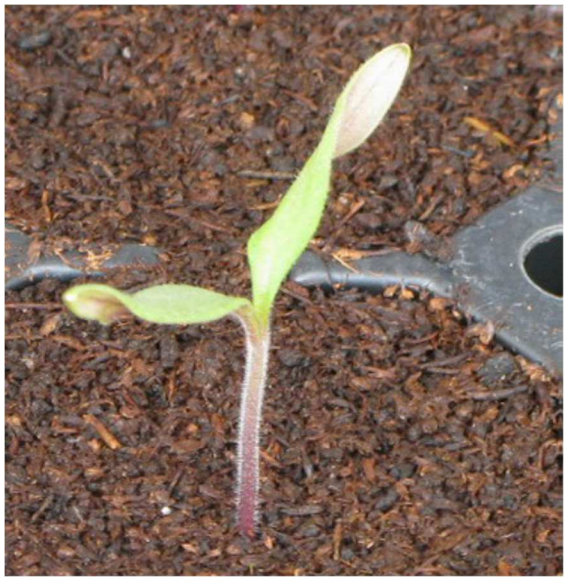
b) Observations on the fruit should be made on mature fruits from the second or higher truss.

c) Observations on the green shoulder and meridian stripes of the fruit should be made on the plant before maturity.

8.2 Explanations for individual characteristics

Ad. 1: Seedling: anthocyanin coloration of hypocotyl

1
absent



9
present

Ad. 2: Plant: height

To be observed after fruit set on 5 nodes.

Ad. 4: Stem: length of internode

The mean length of the internodes between the 1st and 4th trusses should be assessed.

Ad. 7: Leaf: size of leaflets

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

Ad. 9: Leaf: glossiness

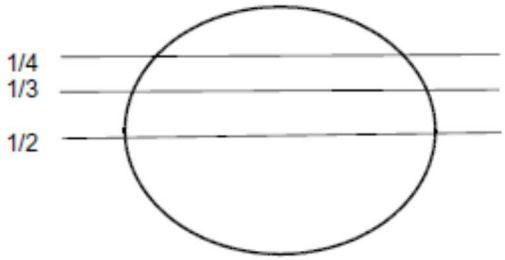
The glossiness of the leaf should be observed in the middle of the plant.

Ad. 10: Leaf: blistering

Caution is required for confusion between blistering and creasing. Blistering is the difference in height of the surface of the leaf between the veins. Creasing is independent form the veins. The blistering should be observed in the middle third of the plant.

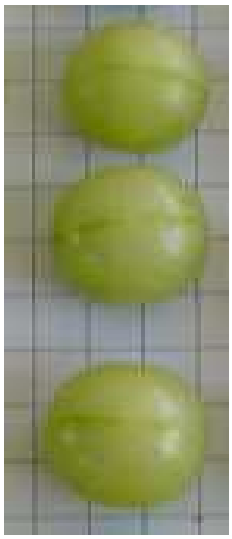
Ad. 12: Fruit: extent of green shoulder

The gene for green shoulder might not be clearly expressed in some conditions.

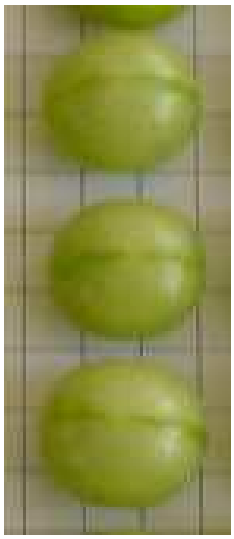


3: small (1/4)
5: medium (1/3)
7: large (1/2)

Ad. 14: Fruit: conspicuousness of meridian stripes



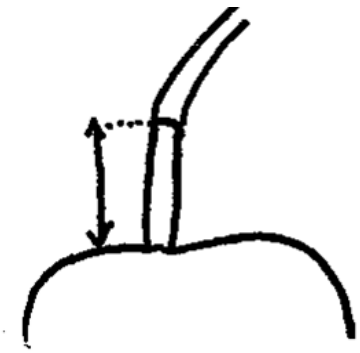
2
weak



3
medium

4
strong

Ad. 15: Pedicel: length

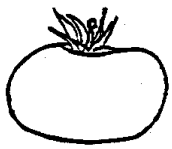


Ad. 16: Fruit: size

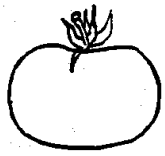
Varieties of certain interspecific crosses for tomato rootstocks may not have viability for production of fruits, or exceptionally produce few very small fruits (note 1).

Ad. 17: Fruit: shape in longitudinal section

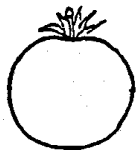
The apex is considered to be the part that is furthest from the stalk attachment.



1
broad oblate



2
narrow oblate



3
circular

4
obovate

Ad. 21: Autonecrosis

Autonecrosis is a necrotic reaction to the presence of incompatible genomes causing older leaves to wither and die.

Ad. 22: Resistance to *Meloidogyne incognita* (Mi)

1.	Pathogen	*Meloidogyne incognita*
2.	Quarantine status	-
3.	Host species	Tomato - *Solanum lycopersicum*
4.	Source of inoculum	GEVES[1] (FR) or INIA – CSIC (ES)[2] or Naktuinbouw (NL[3])
5.	Isolate	non-resistance breaking
6.	Establishment isolate identity	use tomato standards
7	Establishment pathogenicity	use susceptible rootstock or tomato standard
8.	Multiplication inoculum	
8.1	Multiplication medium	living plant
8.2	Multiplication variety	susceptible variety, preferably resistant to powdery mildew
8.3	Plant stage at inoculation	2nd leaf stage
8.5	Inoculation method	deposit of piece of inoculated roots in soil (around 5-10g near each plant, to adapt depending on the population aggressivity)
8.6	Harvest of inoculum	6 to 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 Remark: knowing that germination in rootstocks might be low and/or irregular it is recommended to sow more seeds to be sure to get at least 30 plants. It is recommended to include in the test, 10 non-inoculated plants, to be able to identify a possible lack of germination or a delay in plant growth, due to the material.
9.2	Number of replicates	at least 2, preferably 3 to allow statistical analysis
9.3	Control varieties	Susceptible: Bruce and (*Solanum lycopersicum*) Casaque Rouge Intermediate resistant: (*Solanum lycopersicum*) Campeon, Tyonic Highly resistant: Emperador

[1] GEVES; matref@geves.fr
[2] INIA; resistencias@inia.es
[3] Naktuinbouw; resistentie@naktuinbouw.nl

9.4	Test design	3 replicates of 10 plants in different trays by variety
9.5	Test facility	greenhouse or climate room
9.6	Temperature	20-26°C, the temperature should be adapted, depending on the aggressiveness of the test, to obtain the expected response of the controls, but should not exceed 26°C. Higher temperatures will cause breakdown of resistance.
9.7	Light	at least 12 h per day
10	Inoculation	
10.1	Preparation inoculum	small pieces of diseased roots mixed with soil
10.2	Quantification inoculum	Quantity of inoculum depends on aggressiveness of test and growing conditions (e.g., between 30g to 60g of inoculated roots for 100 plants in a tray of 45*30 cm containing approximately 5.5 kg of substrate); galls should be homogeneously mixed with soil.
10.3	Plant stage at inoculation	seed
10.4	Inoculation method	Seeds are sown in non-inoculated soil and inoculation of soil and inoculation of soil is done after sowing when plantlets are at cotyledon stage.
10.7	End of test	28 to 45 days after inoculation depending on test conditions (temperature, season)
11.	Observations	
11.1	Method	root inspection per plant
11.2	Observation scale	

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

11.3	Validation of test	Validation on controls. Expected reactions of controls: Susceptible control: most plants at classes 3 and 4. Highly resistant: most plants at classes 0 and 1. Intermediate resistant: clearly different from other controls with majority of plants around class 2.
12.	Interpretation of data in terms of UPOV characteristic states	[1] Susceptible: variety very similar to susceptible control [2] Intermediate resistant: variety very similar to intermediate resistant control [3] Highly resistant: variety very similar to highly resistant control If results are not clear, statistical analysis is advised. If significantly different from the controls, a retest is advised to check if the result is stable.
13.	Critical control points	Avoid overwatering. This may result in rotting of roots. In case of aggressive test, decrease the quantity of inoculum.

Ad. 23: Resistance to *Verticillium* sp. (Va and Vd)

1. Pathogen.....	*Verticillium dahliae* or *Verticillium albo-atrum* (see note below)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw (NL[4]) or GEVES[5] (FR)
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 10^6 per ml
8.8 Shelf life/viability inoculum.....	1 d at 4°C
9. Format of the test	
9.1 Number of plants per genotype	35 seeds for 24 plants
9.2 Number of replicates.....	1 replicate
9.3 Control varieties	
Susceptible:	(*Solanum lycopersicum*) Flix, Marmande verte, Clarion, Santonio, Anabel
Resistant:	(*Solanum lycopersicum*) Monalbo, Elias, Monalbo x Marmande verte, Daniela, Marmande VR
9.4 Test design.....	20 plants inoculated at least, 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 inoculum.....	count spores, adjust to 10^6 per ml
10.3 Plant stage at inoculation	cotyledon to third leaf
10.4 Inoculation method.....	roots are immersed for 4 to 15 min in spore suspension
10.7 Final observations	14-33 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. Standards near borderline R/S will help to compare between laboratories.
12. Interpretation of test results in comparison with control varieties	
absent	[1] severe symptoms
present	[9] mild or no symptoms
13. Critical control points	All symptoms may be present in resistant varieties, but the severity will be distinctly less than in susceptible varieties. Usually resistant varieties will show significantly less growth retardation than susceptible varieties

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

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

1.	Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[6] (NL), GEVES[7] (FR) or INIA[8] (ES)
5.	Isolate	race 0EU/1US (e.g. strains Orange 71 or PRI 20698 or Fol 071) race 1EU/2US (e.g. strains 4152 or PRI40698 or RAF 70) race 2EU/3US (e.g. strain Fol029)
6.	Establishment isolate identity	use differential varieties (see ISF website: https://worldseed.org/)
7.	Establishment pathogenicity	on susceptible tomato varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar, Medium "S" of Messiaen
8.4	Inoculation medium	water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
8.6	Harvest of inoculum	filter through double muslin cloth
8.7	Check of harvested inoculum	spore count; adjust to 10^6 per ml
8.8	Shelflife/viability inoculum	4-8 h, keep cool to prevent spore germination
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	1 replicate
9.3.1	Control varieties for the test with race 0EU/1US	
	Susceptible	(*Solanum lycopersicum*) Marmande, Marmande verte, Resal
	Resistant	Emperador, Colosus and (*Solanum lycopersicum*) "Marporum x Marmande verte", Motelle, Gourmet, Mohawk, Ranco, Tradiro
9.3.2	Control varieties for the test with race 1EU/2US	
	Susceptible	(*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma, Marporum, Ranco
	Resistant	Emperador, Colosus and (*Solanum lycopersicum*) Tradiro, Odisea, "Motelle x Marmande verte"
9.3.3	Control varieties for the test with race 2EU/3US	
	Susceptible	Emperador and (*Solanum lycopersicum*) Marmande verte, Motelle, Marporum. Susceptible rootstocks are generally less susceptible than susceptible *Solanum lycopersicum* varieties. The susceptible rootstock variety Emperador must be included as control.
	Resistant	Colosus and (*Solanum lycopersicum*) Tributes, Murdoch, "Marmande verte x Florida"
9.4	Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5	Test facility	glasshouse or climate room
9.6	Temperature	24-28°C (severe test, with mild isolate) 20-24°C (mild test, with severe isolate)
9.7	Light	12 hours per day or longer
9.8	Season	all seasons
9.9	Special measures	slightly acidic peat soil is optimal; keep soil humid but avoid water stress
10.	Inoculation	
10.1	Preparation inoculum	aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
10.2	Quantification inoculum	spore count, adjust to 10^6 spores per ml, lower concentration for a very aggressive isolate

[6] Naktuinbouw: resistentie@naktuinbouw.nl
[7] GEVES: matref@geves.fr
[8] INIA: resistencias@inia.es

10.3	Plant stage at inoculation	10-18 d, cotyledon to first leaf
10.4	Inoculation method	roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
10.7	Final observations	14-21 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon

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	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]	distribution of plants in the classes comparable with the susceptible controls
	present .....[9]	distribution of plants in the classes comparable with the resistant controls.
13.	Critical control points	Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature.

Ad. 25: 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 (NL[9]) or GEVES[10] (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 plants
9.2 Number of replicates.....	1 replicate

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

9.3 Control varieties
Susceptible: ..... Kemerit and (*Solanum lycopersicum*) Motelle, Moneymaker
Resistant: ..... Emperador and (*Solanum lycopersicum*) 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 inocula..... 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 test results in comparison with control varieties

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

Ad. 26: Resistance to *Passalora fulva* (Pf) ()

1.	Pathogen	*Passalora fulva*
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[11] (NL) or GEVES[12] (FR)
5.	Isolate	Race 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	Shelflife/viability inoculum	4 hours, keep cool
9.	Format of the test	
9.1	Number of plants per genotype	more than 20 plants
9.2	Number of replicates	1 replicate
9.3	Control varieties	
	Susceptible	King Kong, (*Solanum lycopersicum*) Monalbo, Moneymaker
	Resistant for race 0:	Bruce, (*Solanum lycopersicum*) Vagabond, Vagabond × IVT 1149, IVT 1154, Purdue
	Resistant for race group A:	Vitalfort, (*Solanum lycopersicum*) Sonato, Purdue, IVT 1154, IVT 1149
	Resistant for race group B:	Bruce, (*Solanum lycopersicum*) Vétomold, IVT 1149, IVT 1154
	Resistant for race group C:	Vitalfort, (*Solanum lycopersicum*) IVT 1154, IVT 1149
	Resistant for race group D:	Bruce, (*Solanum lycopersicum*) Vétomold, IVT 1154
	Resistant for race group E:	Vitalfort, (*Solanum lycopersicum*) 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

[11] Naktuinbouw: resistentie@naktuinbouw.nl
[12] Geves: matref@geves.fr

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 fully closed 3-4 days after inoculation and after that partly closed (66% until 80%, 24h per day), until end
10.	Inoculation	
10.1	Preparation inoculum	prepare evenly colonized plates, e.g. 1 for 36 plants; remove spores from plate by scraping with water with Tween20; filter through double muslin cloth
10.2	Quantification inoculum	count spores; adjust to 10^5 spores per ml or more
10.3	Plant stage at inoculation	19-20 d (incl. 12 d at 24°), 2-3 leaves
10.4	Inoculation method	spray on dry leaves
10.7	Final observations	14 days after inoculation; when susceptible control does not show clear symptoms the test may be prolonged until for example 18 days after inoculation
11.	Observations	
11.1	Method	visual inspection of abaxial side of inoculated leaves
11.2	Observation scale	Symptom: velvety, white spots
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of data in terms of UPOV characteristic states	
	[1] absent	symptoms
	[9] present	no symptoms
13.	Critical control points	Pf spores have a variable size and morphology. Small spores are also viable. Fungal plates will gradually become sterile after 6-10 weeks and repeated subculturing. Do not subculture more often than strictly necessary for multiplication. Store good culture at -80°C. Excessively high humidity may cause rugged brown spots on all leaves. These are not to be considered as off-types.

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

Resistance to strain 0, 1 and 2 to be tested in a bio-assay (method i) and/or in a DNA marker test (method ii).

(i) Bio-assay

1.	Pathogen	Tomato mosaic virus
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[13] (NL) or GEVES[14] (FR)
5.	Isolate	Strain 0 (e.g. isolate INRA Avignon 6-5-1-1), strain 1 and strain 2
6.	Establishment isolate identity	genetically defined tomato standards Mobaci (Tm1), Moperou (Tm2), Momor ($Tm2^2$)
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

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

8.8	Shelflife/viability inoculum	fresh>1 day, desiccated>1year
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	1 replicate
9.3	Control varieties	
	Susceptible	(*Solanum lycopersicum*) Marmande, Monalbo
	Resistant for ToMV: 0 and 2	(*Solanum lycopersicum*) Mobaci
	Resistant for ToMV: 0 and 1	(*Solanum lycopersicum*) Moperou
	Resistant with necrosis	(*Solanum lycopersicum*) "Monalbo x Momor"
	Resistant	(*Solanum lycopersicum*) Gourmet
9.4	Test design	blank treatment with PBS and carborundum or similar buffer
9.5	Test facility	glasshouse or climate room
9.6	Temperature	24 to 26°C
9.7	Light	12 hours or longer
9.8	Season	symptoms are more pronounced in summer
10.	Inoculation	
10.1	Preparation inoculum	1 g leaf with symptoms with 10 ml PBS or similar buffer homogenize, add carborundum to buffer (1 g/30ml)
10.3	Plant stage at inoculation	cotyledons or 2 leaves
10.4	Inoculation method	gentle rubbing
10.7	Final observations	11-21 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	symptoms of susceptibility: mosaic in top, leaf malformation symptoms of resistance (based on hypersensitivity): local necrosis, top necrosis, systemic necrosis
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
	Remark: in some heterozygous varieties a variable proportion of plants may have severe systemic necrosis or some necrotic spots while the other plants have no symptoms. This proportion may vary between experiments.	
12.	Interpretation of test results in comparison with control varieties	
	absent.....[1]	symptoms of susceptibility
	present..... [9]	no symptoms, or symptoms of hypersensitive resistance
13.	Critical control points Temperature and light may influence the development of necrosis. More light means more necrosis. At temperatures above 26°C the resistance may break down. Resistant heterozygous varieties may have symptomless plants and plants with severe necrosis; in spite of apparent segregation the sample may be evaluated as uniform for resistance. Note: Strain INRA Avignon 6-5-1-1 is recommended for ToMV: 0. This strain causes a striking yellow Aucuba mosaic.	

(ii) DNA marker test

Resistance gene Tm2 gives resistance to ToMV. Gene Tm2 has two dominant alleles for resistance: allele Tm2 is always associated with resistance to strain 0 and 1, allele $Tm2^2$ is always associated with resistance to strain 0, 1 and 2. The presence or absence of both alleles for resistance 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	$Tm2/2^2$
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 allele for susceptibility or resistance	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 allele for susceptibility tm2 present: (*Solanum lycopersicon*) Moneymarker homozygous allele for resistance Tm2 present: (*Solanum lycopersicon*) Moperou homozygous allele for resistance $Tm2^2$ present: Emperador
6.	PCR conditions	1. Initial denaturation step at 94°C for 3 minutes 2. 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes 3. Final extension step of 72°C for 10 minutes
8.	Interpretation of test results	The presence of the alleles tm2, Tm2, $Tm2^2$ lead to different interpretation for characteristics 27.1, 27.2 and 27.3, see table. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the variety is resistant due to another mechanism like 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
27.1 Strain 0	[1] absent	[9] resistant	[9] resistant
27.2 Strain 1	[1] absent	[9] resistant	[9] resistant
27.3 Strain 2	[1] absent	[1] absent	[9] resistant

Ad. 28: Resistance to *Pyrenochaeta lycopersici* (Pl)

1.	Pathogen	*Pyrenochaeta lycopersici*
2.	Quarantine status	No
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	GEVES (FR) matref@geves.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: (*Solanum lycopersicum*) Marmande verte Resistant: Emperador and (*Solanum lycopersicum*) Garance
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
10.2	Quantification inoculum	-
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

Ad. 29: 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[15] (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 ..... at least 20 plants
9.2 Number of replicates ..... 1 replicate
9.3 Control varieties
Susceptible:..... (*Solanum lycopersicum*) Monalbo
Resistant: ..... Body and (*Solanum lycopersicum*) 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 Tween
The spore suspension is sieved through a double layer of muslin
10.2 Quantification inoculum..... 5.10^3 - 10^5 spores per ml

[15] GEVES; matref@geves.fr

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 test results in comparison with control varieties
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.*

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

(i) agro-inoculation method

1.	Pathogen	Tomato yellow leaf curl virus (TYLCV) IL strain. (See note below)
2.	Quarantine status	yes (see 13.)
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Dr. Eduardo R. Bejarano, Plant Genetics Laboratory, IHSM-UMA-CSIC[16]
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)[17]
8.6	Harvest of inoculums	
8.7	Check of harvested inoculum	
8.8	Shelflife/viability inoculum	*A. tumefaciens* stocks are maintained frozen at -80ºC in 15-20% glycerol for long term storage. Cultures to be stored are typically started from a single colony and grown in 5 ml YEP +2.5 µl kanamycin (100mg/ml) during 48 h at 28°C.
9.	Format of the test	
9.1	Number of plants per genotype	20
9.2	Number of replicates	2
9.3	Control varieties	Susceptible: (*Solanum lycopersicum*) Moneymaker, Marmande Resistant: (*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk
9.4	Test design	
9.5	Test facility	Glasshouse or climatic chamber with permission to confined use of LMO/GMO, confinement level 1 (N-1)[17]
9.6	Temperature	23-25°C

[16] Source of inoculum: IHSM-UMA-CSIC, edu_rodri@uma.es or INIA, resistencias@inia.es
[17] 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.

9.7	Light	16 h
9.8	Season	
9.9	Special measures	Permission to confined use of LMO/GMO, at least level 1 (N-1)[17]
10.	Inoculation	
10.1	Preparation inoculum	Streak the surface of the frozen *A. tumefaciens* stock tube and submerge in 5 ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 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 inoculums	Dissolve in sterile deionize water to a final OD_{600} of 1.
10.3	Plant stage at inoculation	3-4th leaf
10.4	Inoculation method	Take up into a 1 ml syringe with a 27-gauge needle and few drops (about 20 µl of the culture) were deposited on 10-15 puncture wounds made with the needle into the stem of test tomato plants. Maintain on ice while inoculating plants.
10.5	First observation	20 days post inoculation
10.6	Second observation	30 dpi
*10.7	Final observations	45 dpi
11.	Observations	
11.1	Method	Visual
11.2	Observation scale	Symptoms: leaf yellowing and curling
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of data in terms of UPOV characteristic states	
	absent.....[1]	severe symptoms
	present.....[9]	no symptoms
13.	Critical control points: TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2. TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).	

(ii) White fly inoculation method

1.	Pathogen	Tomato yellow leaf curl virus (TYLCV) IL strain
2.	Quarantine status	yes (see 13.)
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Spain[18]
5.	Isolate	TYLCV-IL La Mayora
8.	Multiplication inoculum	White flies
8.6	Harvest of inoculums	
9.	Format of the test	
9.1	Number of plants per genotype	20
9.2	Number of replicates	Two replicates
9.3	Control varieties	
	Resistant	TY 20, Anastasia, Mohawk
	Susceptible	(*Solanum lycopersicum*) Moneymaker, Marmande
	Resistant	(*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk
9.5	Test facility	greenhouse/plastic tunnel

[18] IHSM-UMA-CSIC, guillamon@eelm.csic.es or INIA, resistencias@inia.es

9.9	Special measures	prevent spread of white-flies
10.	Inoculation	
10.3	Plant stage at inoculation	2-4 weeks
10.4	Inoculation method	vector (Bemisia white-flies carrying TYLCV-IL)
10.7	Final observations	1-2 months after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Symptoms: leaf yellowing and curling
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of data in terms of UPOV characteristic states	
	absent..... [1]	severe symptoms
	present.....[9]	no or mild symptoms
13.	Critical control points: TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).	

Ad. 31: 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 [19] (NL), GEVES [20] (FR)
5.	Isolate	race 0, preferably a thrips-transmission deficient variant
7.	Establishment pathogenicity	biotest
8.	Multiplication inoculum	
8.6	Harvest of inoculum	symptomatic leaves may be stored at -70°C
9.	Format of the test	
9.1	Number of plants per genotype	20 plants
9.2	Number of replicates	1 replicate
9.3	Control varieties	
	Susceptible	(*Solanum lycopersicum*) Monalbo, Momor, Montfavet H 63.5
	Resistant	Enpower and (*Solanum lycopersicum*) Tsunami, Bodar, Mospomor, Lisboa
9.5	Test facility	glasshouse or climatic chamber
9.6	Temperature	20°C
9.7	Light	12 hours or longer
9.9	Special measures	prevent or combat thrips
10.	Inoculation	
10.1	Preparation inoculum	press symptomatic leaves in ice-cold buffer 0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite or similar buffer option: sieve the leaf sap through double muslin
10.3	Plant stage at inoculation	one or two expanded leaves

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

10.4	Inoculation method	mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10° C
10.7	Final observations	7-21 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	symptoms: top mosaic, bronzing, various malformations, necrosis
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of test results in comparison with control varieties	
	absent.....[1]	symptoms
	present.....[9]	no symptoms
13.	Critical control points TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabaci* and Western flower thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties carrying the resistance gene Sw-5.	

(ii) DNA marker test

Dominant resistance gene Sw-5 is always associated with resistance to TSWV strain 0. The presence or absence of the allele for resistance can be detected by the co-dominant marker 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	Allele for susceptibility	Sw5-Vat1-F: 5'-ACAACATCAAACAATGTTAGCC-3' Sw5-Vat2-F: 5'-CATCAAACAATGCAGTTAGCC-3'
3.2	Allele for resistance	Sw5-Res-F: 5'-ATCAACCAATACAGCCTAACC-3
3.3	Universal reverse	Sw5-universal-R: 5'-TTTCTCCCTGCAAGTTCACC-3'
3.4	Allele specific probes	Sw5-Sus1: 5'-VIC-TACATTATGAAGGGTTAACAAG-MGB-NFQ-3' Sw5-Sus2: 5'-6FAM-ACAACAGAGGGTTAACAAGTTTAGG-BHQ1-3' Sw5-Res: 5'-TEXAS RED-TGGGCGAAAATCCCAACAAG-BHQ2-3'
4.	Format of the test	
4.1	Number of plants per genotype	at least 20 plants
4.2	Control varieties	homozygous allele 1 for susceptibility present: Emperador homozygous allele 2 for susceptibility present: (*Solanum lycopersicum*) Mountain Magic homozygous allele for resistance present: Enpower
6.	PCR conditions	1. Initial denaturation step 10 min 95 °C 2. 40 cycles 15 sec 95 °C and 1 min 60°C. Every cycle ends with a plate reading.
8.	Interpretation of test results	
	absent.....[1]	allele(s) for susceptibility present and allele for resistance absent
	present.....[9]	allele for resistance 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 variety is resistant due to another mechanism.

Ad. 32: Resistance to *Oidium neolycopersici* (On)

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	3 weeks
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 plants
9.2 Number of replicates	1 replicate
9.3 Control varieties	
Susceptible:	(*Solanum lycopersicum*) Momor, Montfavet H 63.5
Resistant:	Multifort and (*Solanum lycopersicum*) 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 test results in comparison with control varieties	
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.

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO/TQ/294/1-Rev.6 – *Solanum habrochaites* S. Knapp & D.M. Spooner – tomato rootstocks

Link to e-TQ:
https://online.plantvarieties.eu/backOfficeFormQuestions?viewFormId=15055&viewFormType=TQ&viewFormLang=EN&speciesIds=SOL11&status=1,2&order=formName