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 lycopersicum L. *x Solanum cheesmaniae* (L. Ridley) Fosberg
Solanum pimpinellifolium L. *x Solanum habrochaites* S. Knapp & D.M. Spooner

TOMATO ROOTSTOCKS

UPOV Code: SOLAN_HAB; SOLAN_LHA; SOLAN_LPE; SOLAN_LCH; SOLAN_PHA

Adopted on 21/04/2020

Entry into force on 01/06/2020

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CPVO-TP/294/1-Rev.4

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.), *Solanum lycopersicum* L. *x Solanum cheesmaniae* (L. Ridley) Fosberg 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.) 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.4.

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. 2 dated 05/04/2017 (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 **01.06.2020**. 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.

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 two 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 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.

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. 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.

4.2.2 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) 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 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 6.1.2

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			short	Big Power	3
			medium	Maxifort	5
			tall	Beaufort	7
3.	**3.**	**VG**	**Stem: anthocyanin coloration of upper third**		
QN		**(a)**	absent or very weak		1
			weak	Arnold	3
			medium	Beaufort	5
			strong	Montezuma	7
4. (+)	**4.**	**VG/MS**	**Stem: length of internode**		
QN		**(a)**	short	Big Force	3
			medium	Maxifort	5
			long	Beaufort	7
5.	**5. (*)**	**VG/MS**	**Leaf: length**		
QN		**(a)**	short		3
			medium	Body	5
			long	Maxifort	7
6.	**6. (*)**	**VG/MS**	**Leaf: width**		
QN		**(a)**	narrow		3
			medium	Body	5
			broad	Emperador	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
7. (+)	**7.**	**VG**	**Leaf: size of leaflets**		
QN		**(a)**	very small		1
			small	Titron	3
			medium	Big Force	5
			large	Beaufort	7
			very large	Hires 1210	9
8.	**8. (*)**	**VG**	**Leaf: intensity of green colour**		
QN		**(a)**	light		3
			medium		5
			dark	Maxifort	7
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	Maxifort	9
12. (+)	**12. (*)**	**VG**	**Fruit: extent of green shoulder**		
QN		**(c)**	small	Big Force	3
			medium		5
			large	Maxifort	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
13.	**13. (*)**	**VG**	**Fruit: intensity of green colour of shoulder**		
QN		**(c)**	light		3
			medium		5
			dark	He-man	7
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
15. (+)	**15.**	**VG/MS**	**Pedicel: length**		
QN		**(b)**	short	Titron	3
			medium	Multifort	5
			long	Beaufort	7
16.	**16. (*)**	**VG**	**Fruit: size**		
QN		**(b)**	not developed or very small	RT303	1
			small	Body, Optifort	3
			medium	Emperador	5
			large	Titron	7
17. (+)	**17. (*)**	**VG**	**Fruit: shape in longitudinal section**		
PQ		**(b)**	broad oblate	He-Wolf	1
			narrow oblate	Gladiator	2
			circular	Maxifort	3
G			obovate		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
G			reddish	Brigeor	4
20.	**20.**	**MG**	**Time of flowering**		
QN			early	He-Man	3
			medium	Body	5
			late	Popeye	7
21. (+)	**21. (*)**	**VG**	**Autonecrosis**		
QL			absent	Maxifort	1
G			present	Body	9
22. (+)	**22. (*)**	**VG**	**Resistance to *Meloidogyne incognita* (Mi)**		
QN			susceptible	Bruce	1
			moderately resistant		2
G			highly resistant	Emperador	3
23. (+)	**23. (*)**	**VG**	**Resistance to *Verticillium* sp. (Va and Vd) - Race 0**		
QL			absent	RTS 119	1
G			present	Big Power	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
24. (+)	**24.**		**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
24.1 (*)	**24.1 (*)**	**VG**	**- Race 0EU/1US**		
QL			absent	RTS 119	1
G			present	Emperador	9
24.2 (*)	**24.2 (*)**	**VG**	**- Race 1EU/2US**		
QL			absent	RTS 119	1
G			present	Emperador	9
24.3 (*)	**24.3 (*)**	**VG**	**- Race 2EU/3US**		
QL			absent	Emperador	1
G			present	Colosus	9
25. (*)	**25. (*)**		**Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)**		
(+)			absent	Kemerit	1
QL			present	Emperador	9
26. (+)	**26.**		**Resistance to *Passalora fulva* (Pf) (ex *Fulvia fulva* (Ff) ex *Cladosporium fulvum*)**		
26.1	**26.1**	**VG**	**- Race 0**		
QL			absent	King Kong	1
			present	Bruce	9
26.2	**26.2**	**VG**	**- Group A**		
QL			absent	King Kong	1
			present	Big Power	9
26.3	**26.3**	**VG**	**- Group B**		
QL			absent	King Kong	1
			present	Bruce	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
26.4	**26.4**	**VG**	**- Group C**		
QL			absent		1
			present	Big Power	9
26.5	**26.5**	**VG**	**- Group D**		
QL			absent	King Kong	1
			present	Bruce	9
26.6	**26.6**	**VG**	**- Group E**		
QL			absent	Bruce, King Kong	1
			present	Big Power	9
27. (+)	**27.**		**Resistance to *Tomato mosaic virus* (ToMV)**		
27.1	**27.1**	**VG/VS**	**- Strain 0**		
QL			absent	RTS 119	1
			present	Emperador	9
27.2	**27.2**	**VG/VS**	**- Strain 1**		
QL			absent		1
			present		9
27.3	**27.3**	**VG/VS**	**- Strain 2**		
QL			absent		1
			present		9
28 (+)	**28.**	**VG**	**Resistance to *Pyrenochaeta lycopersici* (Pl)**		
QL			absent		1
G			present	Emperador	9
29.	**29.**	**VG**	**Resistance to *Stemphylium* spp. (Ss)**		
(+)			absent	Big Power	1
QL			present	Body	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
30.	**30.**	**VG**	**Resistance to *Tomato yellow leaf curl virus* (TYLCV)**		
(+)			absent	Big Power	1
QL			present		9
31.	**31.**	**VG/VS**	**Resistance to *Tomato spotted wilt virus* (TSWV) – strain 0**		
(+)			absent	Big Power	1
QL			present	Enpower	9
32.	**32.**	**VG**	**Resistance to *Oidium neolycopersici* (On)**		
(+)			absent		1
QL			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

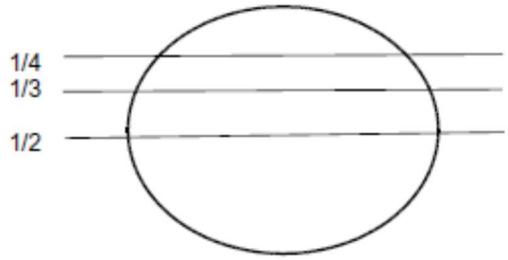
The glossiness of the leaf should be observed in the middle of the plant.

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.

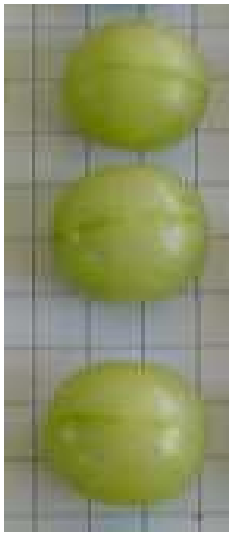
Ad. 12: Fruit: extent of green shoulder

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

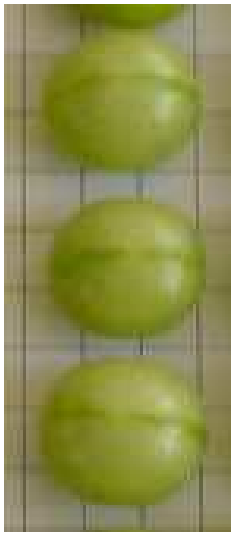


3: small (1/4)
5: medium (1/3)
7: large (1/2)

Ad. 14: Fruit: conspicuousness of meridian stripes



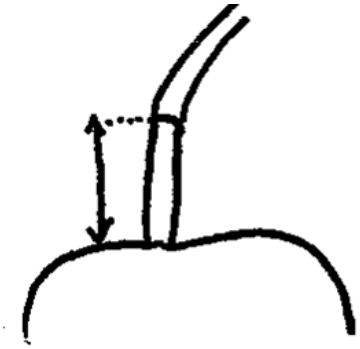
2
weak



3
medium

4
strong

Ad. 15: Pedicel: length



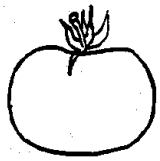
Ad. 16: Fruit: size

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).

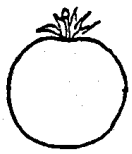
Ad. 17: Fruit: shape in longitudinal section

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*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw (NL[1]) or GEVES[2] (FR)
5. Isolate	non-resistance breaking
6. Establishment isolate identity	use rootstock or 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.....	see 10.3
8.5 Inoculation method	see 10.4
8.6 Harvest of inoculum	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
8.8 Shelf life/viability inoculum.....	1 day
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:.....	Bruce and (*Solanum lycopersicum*) Clairvil, Casaque Rouge
Moderately resistant:.....	(*Solanum lycopersicum*) Campeon,
Highly resistant:.....	Emperador and (*Solanum lycopersicum*) "Anahu x Casaque Rouge", Anahu, Anabel
9.4 Test design.....	include standard varieties
9.5 Test facility.....	greenhouse or climate room
9.6 Temperature.....	not over 28° C
9.7 Light	at least 12 h per day
10. Inoculation	
10.1 Preparation inoculum	small pieces of diseased root mixed with soil mix soil and infested root pieces
10.2 Quantification inoculum.....	soil: root ratio = 8:1, or depending on experience
10.3 Plant stage at inoculation	seed, or cotyledons
10.4 Inoculation method.....	plants are sown in infested soil or contamination of soil after sowing when plantlets are at cotyledon stage
10.7 Final observations	28 to 45 days after inoculation

[1] Naktuinbouw; resistentie@naktuinbouw.nl
[2] GEVES; matref@geves.fr

11. Observations
11.1 Method ..... root inspection
11.2 Observation scale ..... Symptoms: Galling, root malformation, growth reduction, plant death
11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls on standards
12. Interpretation of test results in comparison with control varieties
To consider that resistant varieties may have a few plants with galls. These are not considered as off-types.
absent (susceptible).....[1] growth strongly reduced, high gall count
intermediate (moderately resistant).[2] medium growth reduction, medium gall count
present (highly resistant).....[3] no growth reduction, no galls
13. Critical control points ..... Avoid rotting of roots; high temperature causes breakdown of resistance

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[3]) or GEVES[4] (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: ..... Big Power and (*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

[3] Naktuinbouw; resistentie@naktuinbouw.nl
[4] 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* L.
4. Source of inoculum	Naktuinbouw (NL[5]), GEVES[6] (FR) or INIA[7] (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) and race 2EU/3US (e.g. strain Fol029). Individual strains may vary in pathogenicity
6. Establishment isolate identity	use differential varieties (see 9.3)
7. Establishment pathogenicity	on susceptible tomato varieties
8. Multiplication inoculum	
8.1 Multiplication medium	Potato Dextrose Agar, Medium "S" of Messiaen
8.4 Inoculation medium	water for scraping agar plates or Czapek-Dox broth 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 Shelf life/viability inoculum	4-8 h, keep cool to prevent spore germination
9. Format of the test	
9.1 Number of plants per genotype	at least 20 plants
9.2 Number of replicates	1 replicate
9.3.1 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
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	greenhouse 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 inocula	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 per ml, Lower concentration for a very aggressive isolate
10.3 Plant stage at inoculation	10-18 d, cotyledon to first leaf
10.4 Inoculation method	roots and hypocotyls are immersed in spore suspension
10.7 Final observations	14-21 days after inoculation
11. Observations	
11.1 Method	visual
11.2 Observation scale	Symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
12. Interpretation of test results in comparison with control varieties	
absent	[1] severe symptoms
present	[9] mild or no symptoms
13. Critical control points	Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature. Standards near borderline R/S will help to compare between labs.

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

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[8]) or GEVES[9] (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
9.3 Control varieties	
Susceptible:	Kermit 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 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

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

Ad. 26: Resistance to *Passalora fulva* (Pf) (ex *Fulvia fulva* (Ff) ex *Cladosporium fulvum*)

1. Pathogen.....	*Fulvia fulva* (ex *Cladosporium fulvum*)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw (NL[10]) or GEVES[11] (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 plants
9.2 Number of replicates.....	1 replicate
9.3 Control varieties	
Susceptible:	King Kong and (*Solanum lycopersicum*) Monalbo, Moneymaker
Resistant for race 0:	Bruce and (*Solanum lycopersicum*) Angela, Estrella, Sonatine, Sonato, Vermone, Vagabond, IVT 1149, Vagabond x IVT 1149, IVT 1154
Resistant for race group A:.....	Big Power and (*Solanum lycopersicum*) Angela, Estrella, Sonatine, Sonato
Resistant for race group B:.....	Bruce and (*Solanum lycopersicum*) Angela, Estrella, Sonatine, Sonato, Vermone
Resistant for race group C:.....	Big Power and (*Solanum lycopersicum*) Angela, Estrella, Sonatine
Resistant for race group D:.....	Bruce and (*Solanum lycopersicum*) Estrella, Sonatine, Vermone
Resistant for race group E:	Big Power and (*Solanum lycopersicum*) Sonatine, Jadviga, Rhianna, IVT 1154
9.5 Test facility.....	glasshouse or climate room
9.6 Temperature.....	day: 22°C, night: 20°C 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 inocula.....	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 10^6 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	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

Excessively high humidity may cause rugged brown spots on all leaves. These are not to be considered as off-types.

13. Critical control points.....	Ff 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 ten.

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

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

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

(i) Bio-assay

1. Pathogen.....	*Tomato mosaic virus*
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw (NL[12]) or GEVES[13] (FR) or INIA[14] (SP, strain 0)
5. Isolate	Strain 0 (e.g. isolate INRA Avignon 6-5-1-1) , strain 1 and strain 2.
6. Establishment isolate identity	genetically defined tomato standards Mobaci (Tm1), Moperou (Tm2), Momor (Tm22)
7. Establishment pathogenicity.....	on susceptible plant
8. Multiplication inoculum	
8.1 Multiplication medium.....	living plant
8.2 Multiplication variety.....	e.g. Moneymaker, Marmande
8.7 Check of harvested inoculum....	option: on Nicotiana tabacum "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum.....	fresh>1 day, desiccated>1year
9. Format of the test	
9.1 Number of plants per genotype	at least 20 plants
9.2 Number of replicates.....	1 replicate
9.3 Control varieties	
Susceptible:	(*Solanum lycopersicum*) Marmande, Monalbo
Resistant for ToMV: 0 and 2	(*Solanum lycopersicum*) Mobaci
Resistant for ToMV: 0 and 1	(*Solanum lycopersicum*) Moperou
Resistant with necrosis.....	(*Solanum lycopersicum*) "Monalbo x Momor"
Resistant.....	(*Solanum lycopersicum*) Gourmet
9.4 Test design.....	blank treatment with PBS and carborundum or similar buffer
9.5 Test facility	glasshouse or climate room
9.6 Temperature.....	22 to 26°C
9.7 Light	12 hours or longer
9.8 Season	symptoms are more pronounced in summer
10. Inoculation	
10.1 Preparation inocula.....	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.

[12] Naktuinbouw; resistentie@naktuinbouw.nl
[13] GEVES; matref@geves.fr
[14] 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 ..... $Tm2/2^2$
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 orresistance allele

..... Outer primer TM2-748F:5'CGGTCTGGGGAAAACAACTCT3'
Outer primer TM2-1256R:5'CTAGCGGTATACCTCCACATCTCC3'
TM2-SNP901misR: 5'GCAGGTTGTCCTCCAAATTTTCCATC3'
TM2-SNP901misF: 5'CAAATTGGACTGACGGAACAGAAAGTT3'

4. Format of the test

4.1 Number of plants per genotype at least 20 plants

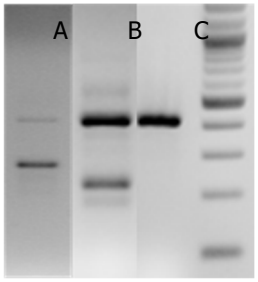
4.2 Control varietieshomozygous susceptible allele tm2 present:

..... (*Solanum lycopersicum*) Moneymaker
resistant allele Tm2 present: (*Solanum lycopersicum*) Moperou
resistant allele Tm22 present: (*Solanum lycopersicum*) Momor, Persica, Campeon

6. PCR conditions..... 1. Initial denaturation step at 94°C for 3 minutes
2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minutes
3. Final extension step of 72°C for 10 minutes

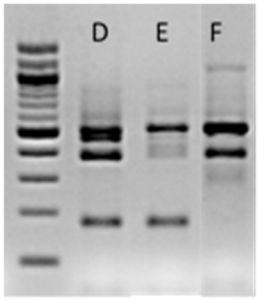
7.1 Observation scale..... Assay 1

A: Control fragment (416bp) and Tm2 fragment (255bp)
B: Control fragment (416bp) and Tm22 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 Tm22 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, Tm22 lead to different interpretation for characteristics 27.1, 27.2 and 27.3, see table. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene Tm1).

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

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

1. Pathogen..... *Pyrenochaeta lycopersici*
3. Host species ..... *Solanum lycopersicum*
4. Source of inoculum ..... -
5. Isolate ..... -
7. Establishment pathogenicity..... biotest
8. Multiplication inoculum
8.1 Multiplication medium..... V8 Agar
8.2 Multiplication variety..... susceptible tomato variety
8.3 Plant stage at inoculation..... seed
8.4 Inoculation medium..... mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1) (10%) or soil mixed with diseased roots cut to small pieces
8.5 Inoculation method ..... sowing, or transplanting at fruit maturity
8.6 Harvest of inoculum ..... diseased roots are harvested after 2-4 months
8.7 Check of harvested inoculum..... visual inspection of lesions on roots
8.8 Shelf life/viability inoculum..... the fungus will not die quickly, but may lose its pathogenicity within a week after isolation on an agar medium
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*) Monfavet H 63.5
Resistant: ..... Emperador and (*Solanum lycopersicum*) Kyndia, Moboglan, Pyrella
9.5 Test facility..... greenhouse or climate room
9.6 Temperature..... day 24°C, night 14°C
9.7 Light ..... 12 hours minimum
10. Inoculation
10.1 Preparation inoculum..... e.g. double-autoclaved mixture of soil with 10% oatmeal added
e.g. Incubate for 10-14 d at 20°C with occasional, repeated turning
10.3 Plant stage at inoculation..... 6 weeks
10.4 Inoculation method..... transplanting into mixture of soil, sand and inoculum (8.4)
or soil mixed with diseased roots cut to small pieces
or naturally infected soil
10.7 Final observations ..... 6-8 weeks after transplanting (flowering plant)
11. Observations
11.1 Method..... visual
11.2 Observation scale ..... Symptoms: brown lesions on roots
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..... The fungus loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

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:	Big Power and (*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 strirred 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
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 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) agroinoculation method

1. Pathogen	*Tomato yellow leaf curl virus* (TYLCV) IL strain. (See note below)
2. Quarantine status	yes (see 13.)
3. Host species	*Solanum lycopersicum* L.
4. Source of inocula	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

[15] GEVES; matref@geves.fr
[16] Source of inoculum; HMS UMA (CSIC) edu_rodri@uma.es; INIA resistencias@inia.es

8.5 Inoculation method	Stem puncture agroinfiltration. Plant agroinoculation is carried out using Agrobacterium tumefaciens transformed with plasmids containing the infectious clones (Morilla, et al. 2005. Phytopathology 95: 1089-1097)
8.6 Harvest of inocula	
8.7 Check of harvested inocula.....	
8.8 Shelflife/viability inocula.....	*A. tumefaciens* stocks are maintained frozen at -80ºC in 15-20% glycerol for long term storage. Cultures to be stored are typically started from a single colony and grown in 5 ml YEP +2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC.
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	2
9.3 Control varieties	
Susceptible:	Big Power, (*Solanum lycopersicum*) Moneymaker, Marmande
Resistant:	(*Solanum lycopersicum*) Delyca, Montenegro, Anastasia, TY20, Mohawk
9.4 Test design.....	
9.5 Test facility.....	Glasshouse or climatic chamber with permission to confined use of use of LMO/GMO, confinement level 1 (N-1)[17]
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 5 ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 28ªC. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28ºC. Centrifuge the saturated culture for 20 min at 3500 rpm and discard supernatant.
10.2 Quantification 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 End of test – Final observation	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).

[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.

(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* L.
4. Source of inoculum	-Spain[18]
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.....	2
9.3 Control varieties	
Susceptible:	Big Power, (*Solanum lycopersicum*) Moneymaker, Marmande
Resistant:	(*Solanum lycopersicum*) 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).

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). In case of a bio-assay, type of observation is VG. In case of a DNA marker test, type of observation is VS.

(i) Bio-assay

1. Pathogen.....	*Tomato spotted wilt virus* (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	strain 0, preferably a thrips-transmission deficient variant
7. Establishment pathogenicity.....	biotest
8. Multiplication inoculum	
8.6 Harvest of inoculum	symptomatic leaves may be stored at -70°C
9. Format of the test	
9.1 Number of plants per genotype	20 plants
9.2 Number of replicates.....	1 replicate
9.3 Control varieties	
Susceptible:	Big Power and (*Solanum lycopersicum*) Monalbo, Momor, Montfavet H 63.5
Resistant:	Enpower and (*Solanum lycopersicum*) Tsunami, Bodar, PI-Mospomor, Lisboa
9.5 Test facility	glasshouse or climatic chamber

[18] IHSM, CSIC guillamon@eelm.csic.es or resistencias@inia.es
[19] Naktuinbouw: resistentie@naktuinbouw.nl
[20] GEVES; matref@geves.fr

9.6 Temperature..... 20°C
9.7 Light ..... 12 hours or longer
9.9 Special measures ..... prevent or combat thrips
10. Inoculation
10.1 Preparation inoculum ..... press symptomatic leaves in ice-cold buffer
0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite or similar buffer
Option: sieve the leaf sap through double muslin
10.3 Plant stage at inoculation ..... one or two expanded leaves
10.4 Inoculation method..... mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10° C
10.7 Final observations..... 7-21 days after inoculation
11. Observations
11.1 Method..... visual
11.2 Observation scale ..... Symptoms: top mosaic, bronzing, various malformations, nocrosis
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

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 varietie ..... s homozygous susceptible allele 1 present:
(*Solanum lycopersicum*) Moneymaker
homozygous susceptible allele 2 present:
(*Solanum lycopersicum*) Mountain Magic
homozygous resistant allele present:
(*Solanum lycopersicum*) Montealto
6. PCR Conditions ..... 1. Initial denaturation step 10 min 95 °C
..... 2. 40 cycles 15 sec 95 °C and 1 min 60°C. Every cycle ends with a plate reading.
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. 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.4