



## **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 15/03/2017**

**Entry into force on 01/01/2017**

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## 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. (*Lycopersicon esculentum* L. (Mill.))).

Rootstocks belonging to *Solanum lycopersicum* L. (*Lycopersicon esculentum* Mill.) or to *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. (*Lycopersicon esculentum* Mill. x *Lycopersicon pimpinellifolium* Mill.) should be covered by the most recent version of the CPVO protocol for tomato TP/44.

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/en/publications/intro\\_dus.htm](http://www.upov.int/en/publications/intro_dus.htm)), its associated TGP documents (<http://www.upov.int/en/publications/tgp/>) and the relevant UPOV Test Guideline TG/294/1 dated 20/03/2013 (<http://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 **01.01.2017**. 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://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette> 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**

#### **Two independent growing cycles**

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

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

### **3.2 Testing Place**

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness"

[http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp\\_9\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_9_1.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 Additional tests**

In accordance with Article 83(3) of Council Regulation No. 2100/94 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, an additional test may be undertaken providing that a technically acceptable test procedure can be devised.

Additional 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) Fruit species and seed propagated agricultural and vegetable species**

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

#### 3.6.2 Living Plant Material

##### **(a) Fruit species and seed propagated agricultural and vegetable species**

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

##### **(b) Vegetatively propagated agricultural and vegetable species and ornamental species**

The examination office 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 examination office.

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

The inventory shall take into account the list of protected varieties and the official, or other, registers of varieties, in particular:

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 examination office 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 examination office 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/export/sites/upov/en/publications/tgp/documents/tgp\\_9\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_9_1.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.

When resistance characteristics are used for assessing distinctness, uniformity and stability, records must be taken under conditions of controlled infection and, unless otherwise indicated, 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

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/export/sites/upov/en/publications/tgp/documents/tgp\\_10\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_10_1.pdf)) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

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/export/sites/upov/en/publications/tgp/documents/tgp\\_11\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_11_1.pdf))

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 ORGANIZATION 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 organize 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 0 (ex 1) (characteristic 24.1)
- h) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 1 (ex 2) (characteristic 24.2)
- i) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 2 (ex 3) (characteristic 24.3)

5.4 If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the examination office shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

## 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.

#### Technical Protocols with asterisked characteristics (only for certain vegetable species)

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

#### 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

### 6.2 Example Varieties

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

### 6.3 Legend

For the CPVO N° column:

G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	– see Chapter 6.1.2
MG, MS, VG, VS		– see Chapter 4.1.5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	

(a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.

For the UPOV N° column:

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

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



## 7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>1.</b>	<b>1.</b>	<b>VG</b>	<b>Seedling: anthocyanin coloration of hypocotyl</b>		
(+)	(*)		absent		1
<b>QL</b>			present	Beaufort	9
<b>2.</b>	<b>2.</b>	<b>VG</b>	<b>Plant: height</b>		
(+)			short	Big Power	3
<b>QN</b>			medium	Maxifort	5
			tall	Beaufort	7
<b>3.</b>	<b>3.</b>	<b>VG</b>	<b>Stem: anthocyanin coloration of upper third</b>		
<b>QN</b>		<b>(a)</b>	absent or very weak		1
			weak	Arnold	3
			medium	Beaufort	5
			strong	Montezuma	7
<b>4.</b>	<b>4.</b>	<b>VG/MS</b>	<b>Stem: length of internode</b>		
(+)		<b>(a)</b>	short	Big Force	3
<b>QN</b>			medium	Maxifort	5
			long	Beaufort	7
<b>5.</b>	<b>5.</b>	<b>VG/MS</b>	<b>Leaf: length</b>		
	<b>(*)</b>	<b>(a)</b>	short		3
<b>QN</b>			medium	Body	5
			long	Maxifort	7
<b>6.</b>	<b>6.</b>	<b>VG/MS</b>	<b>Leaf: width</b>		
	<b>(*)</b>	<b>(a)</b>	narrow		3
<b>QN</b>			medium	Body	5
			broad	Emperador	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>7.</b>	<b>7.</b>	<b>VG</b>	<b>Leaf: size of leaflets</b>		
(+)		(a)	very small		1
<b>QN</b>			small	Titron	3
			medium	Big Force	5
			large	Beaufort	7
			very large	Hires 1210	9
<b>8.</b>	<b>8.</b>	<b>VG</b>	<b>Leaf: intensity of green colour</b>		
	(*)	(a)	light		3
<b>QN</b>			medium		5
			dark	Maxifort	7
<b>9.</b>	<b>9.</b>	<b>VG</b>	<b>Leaf: glossiness</b>		
(+)		(a)	weak	Montezuma	1
<b>QN</b>			medium	Titron	2
			strong	Maxifort	3
<b>10.</b>	<b>10.</b>	<b>VG</b>	<b>Leaf: blistering</b>		
(+)		(a)	weak	Montezuma	1
<b>QN</b>			medium	Emperador	2
			strong	Body	3
<b>11.</b>	<b>11.</b>	<b>VG</b>	<b>Fruit: green shoulder</b>		
<b>QL</b>	(*)	(c)	absent		1
<b>G</b>			present	Maxifort	9
<b>12.</b>	<b>12.</b>	<b>VG</b>	<b>Fruit: extent of green shoulder</b>		
(+)	(*)	(c)	small	Big Force	3
<b>QN</b>			medium		5
			large	Maxifort	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
13.	13.	VG  (*) (c)	<b>Fruit: intensity of green colour of shoulder</b>			
			light		3	
			medium		5	
QN			dark	He-man	7	
14.	14.	VG  (c)	<b>Fruit: conspicuousness of meridian stripes</b>			
			(+)	very weak	He Wolf	1
			QN	weak	Popeye	2
				medium	Body	3
				strong	Vigomax	4
			very strong		5	
15.	15.	VG/MS  (b)	<b>Pedicel: length</b>			
			(+)	short	Titron	3
			QN	medium	Multifort	5
			long	Beaufort	7	
16.	16.	VG  (*) (b)	<b>Fruit: size</b>			
				not developed or very small	RT303	1
				small	Body, Optifort	3
			QN	medium	Emperador	5
			large	Titron	7	
17.	17.	VG  (*) (b)	<b>Fruit: shape in longitudinal section</b>			
			(+)	broad oblate	He-Wolf	1
			PQ	narrow oblate	Gladiator	2
				circular	Maxifort	3
			obovate		4	
18.	18.	VG/MS  (*) (b)	<b>Fruit: number of locules</b>			
				only two	Maxifort	1
QN			two and three		2	

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
19.   PQ	19.  (* (b)	VG  (b)	<b>Fruit: colour at maturity</b>			
			green	Big Force	1	
			yellowish	Vigomax	2	
			orangish	Titron	3	
			reddish	Brigeor	4	
20.  QN	20.	MG	<b>Time of flowering</b>			
			early	He-Man	3	
			medium	Body	5	
			late	Popeye	7	
21. (+)	21. (* (*)	VG	<b>Autonecrosis</b>			
			absent	Maxifort	1	
			present	Body	9	
22. (* (+)	22. (* (*)	VG	<b>Resistance to <i>Meloidogyne incognita</i> (Mi)</b>			
			susceptible	Bruce	1	
			moderately resistant		2	
			highly resistant	Emperador	3	
23. (* (+)	23. (* (*)	VG	<b>Resistance to <i>Verticillium</i> sp. (Va and Vd) - Race 0</b>			
			absent		1	
			present	Big Power	9	
24. (+)	24. (+)	VG	<b>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)</b>			
			<b>- Race 0 (ex 1)</b>			
			absent		1	
24.1 (* (*)	24.1 (* (*)	VG				
			present		9	

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>24.2</b> (* )	<b>24.2</b> (* )	<b>VG</b>	<b>- Race 1 (ex 2)</b>		
<b>QL</b>			absent		1
<b>G</b>			present		9
<b>24.3</b> (* )	<b>24.3</b> (* )	<b>VG</b>	<b>- Race 2 (ex 3)</b>		
<b>QL</b>			absent	Emperador	1
<b>G</b>			present	Colosus	9
<b>25.</b> (* )	<b>25.</b> (* )		<b>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i> (Forl)</b>		
<b>(+)</b>			absent	Kemerit	1
<b>QL</b>			present	Emperador	9
<b>26.</b> (+ )	<b>26.</b>		<b>Resistance to <i>Fulvia fulva</i> (Ff) (ex <i>Cladosporium fulvum</i>)</b>		
<b>26.1</b>	<b>26.1</b>	<b>VG</b>	<b>- Race 0</b>		
<b>QL</b>			absent	King Kong	1
			present	Bruce	9
<b>26.2</b>	<b>26.2</b>	<b>VG</b>	<b>- Group A</b>		
<b>QL</b>			absent	King Kong	1
			present	Big Power	9
<b>26.3</b>	<b>26.3</b>	<b>VG</b>	<b>- Group B</b>		
<b>QL</b>			absent	King Kong	1
			present	Bruce	9
<b>26.4</b>	<b>26.4</b>	<b>VG</b>	<b>- Group C</b>		
<b>QL</b>			absent		1
			present	Big Power	9
<b>26.5</b>	<b>26.5</b>	<b>VG</b>	<b>- Group D</b>		
<b>QL</b>			absent	King Kong	1
			present	Bruce	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>26.6</b>	<b>26.6</b>	<b>VG</b>	<b>- Group E</b>		
<b>QL</b>			absent	Bruce, King Kong	1
			present	Big Power	9
<b>27. (+)</b>	<b>27.</b>		<b>Resistance to Tomato mosaic virus (ToMV)</b>		
<b>27.1</b>	<b>27.1</b>	<b>VG</b>	<b>- Strain 0</b>		
<b>QL</b>			absent		1
			present	Emperador	9
<b>27.2</b>	<b>27.2</b>	<b>VG</b>	<b>- Strain 1</b>		
<b>QL</b>			absent		1
			present		9
<b>27.3</b>	<b>27.3</b>	<b>VG</b>	<b>- Strain 2</b>		
<b>QL</b>			absent		1
			present		9
<b>28 (+)</b>	<b>28.</b>	<b>VG</b>	<b>Resistance to <i>Pyrenochaeta lycopersici</i> (PI)</b>		
<b>QL</b>			absent	Zaralto	1
<b>G</b>			present	Emperador	9
<b>29. (+)</b>	<b>29.</b>	<b>VG</b>	<b>Resistance to <i>Stemphylium</i> spp. (Ss)</b>		
<b>QL</b>			absent	Big Power	1
			present	Body	9
<b>30. (+)</b>	<b>30.</b>	<b>VG</b>	<b>Resistance to Tomato yellow leaf curl virus (TYLCV)</b>		
<b>QL</b>			absent	Big Power	1
			present		9
<b>31. (+)</b>	<b>31.</b>	<b>VG</b>	<b>Resistance to Tomato spotted wilt virus (TSWV)</b>		
<b>QL</b>			absent	Big Power	1
			present	Enpower	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
32.	32.	VG	<b>Resistance to <i>Oidium neolycopersici</i> (On)</b>		
(+)			absent		1
QL			present	Multifort	9

## 8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

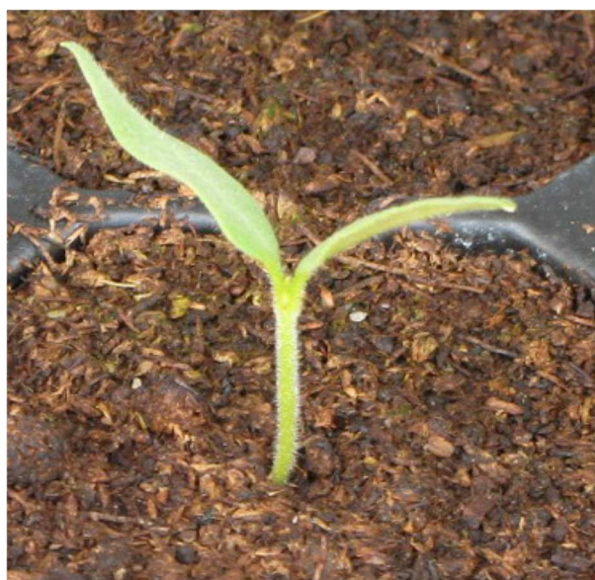
### 8.1 Explanations covering several characteristics

Characteristics containing the following key in the first 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: Plant: height

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 leaflet 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.

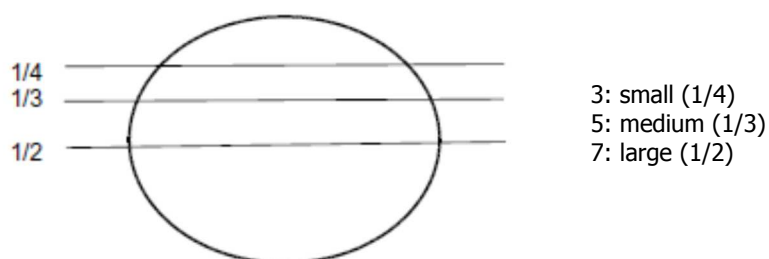


Ad. 10: Leaf: blistering

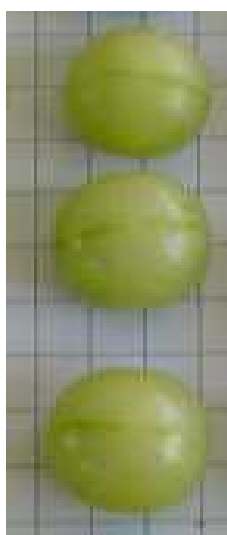
Caution is required for confusion between blistering and creasing. Blistering is the difference in height of the surface of the leaf between the veins. Creasing is independent from the veins. The blistering should be observed in the middle third of the plant

Ad. 12: Fruit: extent of green shoulder

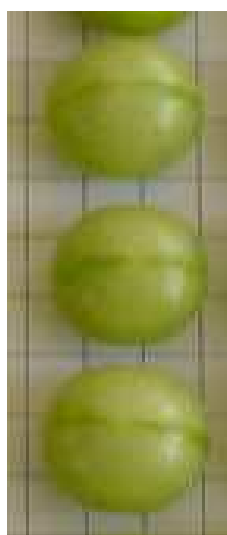
The gene for green shoulder might not be clearly expressed in some conditions



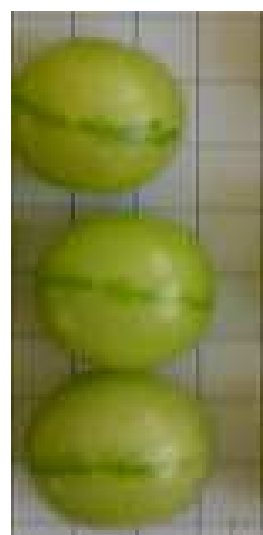
Ad. 14: Fruit: conspicuousness of meridian stripes



2  
weak

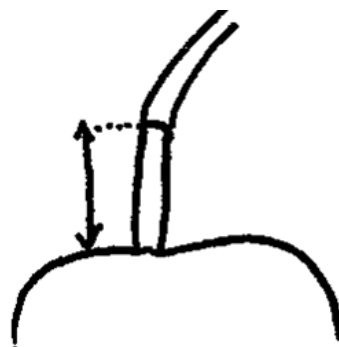


3  
medium



4  
strong

Ad. 15: Pedicel: length

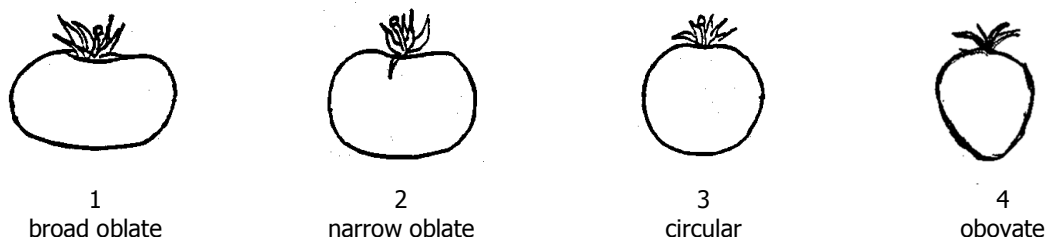


Ad. 16: Fruit: size

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.



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 .....	<i>Meloidogyne incognita</i>
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	Naktuinbouw (NL <sup>1</sup> ) or GEVES <sup>2</sup> (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 ( <i>Solanum lycopersicum</i> ) Clairvil, Casaque Rouge
Moderately resistant: .....	( <i>Solanum lycopersicum</i> ) Campeon,
Highly resistant: .....	Emperador and ( <i>Solanum lycopersicum</i> ) "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

<sup>1</sup> Naktuinbouw; resistentie@naktuinbouw.nl

<sup>2</sup> GEVES; Valerie.grimault@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 falls. 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<sup>3</sup>) or GEVES<sup>4</sup> (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 inoculums .. spore count; adjust to 10<sup>6</sup> 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: ..... | ( <i>Solanum lycopersicum</i> ) Flix, Marmande verte, Clarion, Santonio, Anabel                              |
| Resistant: .....   | Big Power and ( <i>Solanum lycopersicum</i> ) 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 inoculums ..... aerated, liquid culture (8.4)
- 10.2 Quantification inoculum ..... count spores, adjust to 10<sup>6</sup> 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

<sup>3</sup> Naktuinbouw; resistantie@naktuinbouw.nl

<sup>4</sup> GEVES; Valerie.grimault@geves.fr

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

1. Pathogen .....	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	Naktuinbouw (NL <sup>5</sup> ) or GEVES <sup>6</sup> (FR)
5. Isolate .....	Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70 and 2 (ex 3) Individual strains may vary in pathogenicity
6. Establishment isolate identity .....	use differential varieties (see 9.3)
7. Establishment pathogenicity .....	on susceptible 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 <sup>6</sup> per ml
8.8 Shelf life/viability inoculum .....	4-8 h, keep cool to prevent spore germination
9. Format of the test	
9.1 Number of plants per genotype	at least 20 plants
9.2 Number of replicates .....	1 replicate
9.3 Control varieties for the test with race 0 (ex 1)	
Susceptible: .....	( <i>Solanum lycopersicum</i> ) Marmande, Marmande verte, Resal
Resistant for race 0 only: .....	( <i>Solanum lycopersicum</i> ) Marporum, Larissa, "Marporum x Marmande verte", Marsol, Anabel
Resistant for race 0 and 1: .....	( <i>Solanum lycopersicum</i> ) Motelle, Gourmet, Mohawk
Control varieties for the test with race 1 (ex 2)	
Susceptible: .....	( <i>Solanum lycopersicum</i> ) Marmande verte, Cherry Belle, Roma
Resistant for race 0 only: .....	( <i>Solanum lycopersicum</i> ) Marporum, Ranco
Resistant for race 0 and 1: .....	( <i>Solanum lycopersicum</i> ) Tradiro, Odisea
Remark:	Ranko is slightly less resistant than Tradiro
Control varieties for the test with race 2 (ex 3)	
Resistant for race 0, 1 and 2: ...	Colosus
9.4 Test design .....	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility .....	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 inoculums .....	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 <sup>6</sup> 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

<sup>5</sup> Naktuinbouw; resistentie@naktuinbouw.nl

<sup>6</sup> GEVES; Valerie.grimault@geves.fr

Ad. 25: Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl)

1. Pathogen .....	<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	Naktuinbouw (NL <sup>7</sup> ) or GEVES <sup>8</sup> (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 <sup>6</sup> 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 ( <i>Solanum lycopersicum</i> ) Motelle, Moneymaker
Resistant: .....	Emperador and ( <i>Solanum lycopersicum</i> ) 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 inoculums .....	aerated culture or scraping of plates
10.2 Quantification inoculum .....	spore count, adjust to 10 <sup>6</sup> 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 pinpoint 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

<sup>7</sup> Naktuinbouw; resistantie@naktuinbouw.nl

<sup>8</sup> GEVES; Valerie.grimault@geves.fr

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

1. Pathogen .....	<i>Fulvia fulva</i> (ex <i>Cladosporium fulvum</i> )
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	Naktuinbouw (NL <sup>9</sup> ) or GEVES <sup>10</sup> (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 ( <i>Solanum lycopersicum</i> ) Monalbo, Moneymaker
Resistant for race 0: .....	Bruce and ( <i>Solanum lycopersicum</i> ) Angela, Estrella, Sonatine, Sonato, Vermone, Vagabond, IVT 1149, Vagabond x IVT 1149, IVT 1154
Resistant for race group A: .....	Big Power and ( <i>Solanum lycopersicum</i> ) Angela, Estrella, Sonatine, Sonato
Resistant for race group B: .....	Bruce and ( <i>Solanum lycopersicum</i> ) Angela, Estrella, Sonatine, Sonato, Vermone
Resistant for race group C: .....	Big Power and ( <i>Solanum lycopersicum</i> ) Angela, Estrella, Sonatine
Resistant for race group D: .....	Bruce and ( <i>Solanum lycopersicum</i> ) Estrella, Sonatine, Vermone
Resistant for race group E: .....	Big Power and ( <i>Solanum lycopersicum</i> ) 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 inoculums .....	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 <sup>6</sup> 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 pinpoint 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.

<sup>9</sup> Naktuinbouw; resistentie@naktuinbouw.nl

<sup>10</sup> GEVES; Valerie.grimault@geves.fr

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

1. Pathogen .....	Tomato mosaic virus
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	Naktuinbouw (NL <sup>11</sup> ) or GEVES <sup>12</sup> (FR)
5. Isolate .....	Strain 0 (e.g. isolate INRA Avignon 6-5-1-1) 1 and 2
6. Establishment isolate identity .....	genetically defined tomato standards Mobaci (Tm1), Moperou (Tm2), Momor (Tm2 <sup>2</sup> )
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 <i>Nicotiana tabacum</i> "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum .....	fresh > 1 day, desiccated > 1 year
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: .....	( <i>Solanum lycopersicum</i> ) Marmande, Monalbo
Resistant for ToMV: 0 and 2 .....	( <i>Solanum lycopersicum</i> ) Mobaci
Resistant for ToMV: 0 and 1 .....	( <i>Solanum lycopersicum</i> ) Moperou
Resistant with necrosis .....	( <i>Solanum lycopersicum</i> ) "Monalbo x Momor"
Resistant .....	( <i>Solanum lycopersicum</i> ) 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 inoculums .....	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

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

1. Pathogen .....	<i>Pyrenochaeta lycopersici</i>
3. Host species .....	<i>Solanum lycopersicum</i>
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: .....	Zaralto and ( <i>Solanum lycopersicum</i> ) Monfavet H 63.5
Resistant: .....	Emperador and ( <i>Solanum lycopersicum</i> ) 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 .....	<i>Stemphylium</i> spp. e.g. <i>Stemphylium solani</i> (see note below)
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	GEVES <sup>13</sup> (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 ( <i>Solanum lycopersicum</i> ) Monalbo
Resistant: .....	Body and ( <i>Solanum lycopersicum</i> ) 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 <sup>3</sup> - 10 <sup>5</sup> 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)

1. Pathogen .....	Tomato yellow leaf curl virus (see note below)
2. Quarantine status .....	yes
3. Host species .....	<i>Solanum lycopersicum</i>
4. Source of inoculum .....	-
5. Isolate .....	-
8. Multiplication inoculum	
8.1 Multiplication medium .....	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: .....	( <i>Solanum lycopersicum</i> ) Montfavet H 63.5
Resistant: .....	( <i>Solanum lycopersicum</i> ) TY 20, Anastasia, Mohawk
9.5 Test facility .....	field with natural disease pressure
9.9 Special measures .....	prevent spread of white-flies

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10. Inoculation		
10.3 Plant stage at inoculation .....	6-12 weeks (adult plants)	
10.4 Inoculation method .....	vector (Bermisia white-flies carrying TYLCV)	
10.7 Final observations .....	1-2 months after inoculation	
11. Observations		
11.1 Method.....	visual	
11.2 Observation scale.....	Symptoms: leaf yellowing and curling	
11.3 Validation of test .....	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls	
12. Interpretation of test results in comparison with control varieties		
absent	[1]	severe symptoms
present	[9]	no or mild symptoms
13. Critical control points.....	TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV)	

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

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

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

1. Pathogen .....	<i>Oidium neolycopersici</i> (Powdery mildew)
3. Host species .....	<i>Solanum lycopersicum</i>
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: .....	( <i>Solanum lycopersicum</i> ) Momor, Montfavet H 63.5
Resistant: .....	Multifort and ( <i>Solanum lycopersicum</i> ) 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 <sup>4</sup> 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 <i>O. neolycopersici</i> 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 <i>O. neolycopersici</i> 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.2