

# PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Solanum lycopersicum L.

## **TOMATO**

UPOV Code: SOLAN\_LYC

**Adopted on 27/02/2013** 

Entry into force on 27/02/2013

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## CPVO-TP/044/4 Rev.

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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 lycopersicum L**. (including rootstocks), as well as to rootstocks belonging to *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L.

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), its associated TGP documents

(http://www.upov.int/en/publications/tgp/) and the relevant UPOV Test Guideline TG/44/11 dated 06/04/2011 (http://www.upov.int/en/publications/tg-rom/tg044/tg\_44\_10.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

## 1.2 Entry into Force

The present protocol enters into force on **27.02.2013**. 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 <u>Informing on problems in the DUS test</u>

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

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/tap/documents/tap 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 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) Fruit species and seed propagated agricultural and vegetable species

The EO 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 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 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 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/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.

#### **Decision standards**

#### 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), quidance 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) 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)

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) Plant: growth type (characteristic 2)
  - b) Leaf: type of blade (characteristic 10)
  - c) Peduncle: abscission layer (characteristic 19)
  - d) Fruit: green shoulder (before maturity) (characteristic 21)
  - e) Fruit: green stripes (before maturity) (characteristic 25)
  - f) Fruit: size (characteristic 26)
  - g) Fruit: shape in longitudinal section (characteristic 28)
  - h) Fruit: number of locules (characteristic 36)
  - i) Fruit: colour (at maturity) (characteristic 37)
  - j) Resistance to Meloidogyne incognita (characteristic 43)
  - k) Resistance to *Verticilium* sp. (Va and Vd) Race 0 (characteristic 44)
  - Resistance to Fusarium oxysporum f. sp. lycopersici Race 0 (ex 1) (characteristic 45.1)
  - m) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 1 (ex 2) (characteristic 45.2)
  - n) Resistance to Tomato mosaic virus (ToMV) Strain 0 (characteristic 48.1)
  - o) Resistance to Tomato spotted wilt virus (TSWV) Race 0 (characteristic 55)

**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 EO 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  $N^{\circ}874/2009$ , to insert additional characteristics and their expressions in respect of a variety.

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

6.1.2 "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

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

Legend: Explanations covering several characteristics

(a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1
 (+) See Explanations on the Table of Characteristics in Chapter 8.

## 7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1. (+)	1.	VG	Seed-propagated varieties only: Seedling: anthocyanin coloration of hypocotyl		
QL			absent	Colt, Heinz 8104, Mogeor, Momorvert, VTM215	1
			present	Montfavet H 63.4, DG-039	9
2.	2. (*)	VG	Plant: growth type		
(+)			determinate	Campbell 1327, Prisca	1
QL G			indeterminate	Marmande VR, Saint-Pierre, San Marzano 2	2
3.	3.	VG/MS	Only varieties with plant growth type determinate: Plant: number of inflorescences on main stem (side shoots to be removed)		
QN			few	Campbell 1327	3
			medium	Montfavet H 63.4	5
			many	Prisca	7
4.	4.	VG	Stem: anthocyanin coloration		
(+)			absent or very weak	Mogeor, Momorvert	1
QN			weak	Montfavet H 63.5	3
			medium	Rondello	5
			strong	Grinta, Nemato	7
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
5.	5.	VG/MS	Only varieties with plant growth type indeterminate: Stem: length of internode		
(+)		(a)	short	Dombito, Manific, Paso, Trend	3
QN			medium	Montfavet H 63.5	5
			long	Berdy, Calimero	7
6.	6.	VG/MS	Only varieties with plant growth type indeterminate: Plant: height		
(+)			very short	Cherry Belle	1
QN			short	Carson, Despina	3
			medium	Brooklyn, Buffalo, Vision	5
			long	Classy, Clarence, Climberly, Massada	7
			very long	Daydream, Minired	9
7.	7.	VG	Leaf: attitude		
	(*)	(a)	erect		1
(+)			semi-erect	Allround, Drakar, Vitador	3
QN			horizontal	Aromata, Triton	5
			semi-drooping	Montfavet H 63.5	7
			drooping	Multolino, Naram, Tibet	9
8.	8.	VG/MS	Leaf: length		
QN	N	(a)	short	Nelson, Red Robin, Tiny Tim	3
			medium	Lorena	5
			long	Montfavet H 63.5	7
9.	9.	VG/MS	Leaf: width		
QN		(a)	narrow	Marmande VR, Red Robin, Tiny Tim	3
			medium		5
			broad	Saint-Pierre	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
10.	10. (*)	VG	Leaf: type of blade		
(+)		(a)	pinnate	Mikado, Pilot, Red Jacket	1
QL G			bipinnate	Lukullus, Saint-Pierre	2
11.	11.	VG	Leaf: size of leaflets		
(+)		(a)	very small	Minitom	1
QN			small	Tiny Tim	3
			medium	Marmande VR, Royesta	5
			large	Daniela, Hynema	7
			very large	Dombo	9
12.	12.	VG	Leaf: intensity of green colour		
QN		(a)	light	Macero II, Poncette, Rossol	3
			medium	Lucy	5
			dark	Allround, Daniela, Lorena, Red Robin	7
13.	13.	VG	Leaf: glossiness		
(+)		(a)	weak	Daniela	3
QN			medium	Marmande VR	5
			strong	Guindilla	7
14.	14.	VG	Leaf: blistering		
(+)		(a)	weak	Daniela	3
QN			medium	Marmande VR	5
			strong	Delfine, Tiny Tim	7
15.	15.	VG	Leaf: attitude of petiole of leaflet in relation to main axis		
(+)		(a)	semi-erect	Blizzard, Marmande VR	3
QN			horizontal	Sonatine	5
			semi-drooping	Montfavet H63.5	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
16.	16.	VG/MS	Inflorescence: type		
(+)			mainly uniparous	Dynamo	1
QN			equally uniparous and multiparous	Harzfeuer	2
			mainly multiparous	Marmande VR	3
17.	17.	VG	Flower: colour		
	(*)		yellow	Exota, Marmande VR	1
QL			orange	Orama, Pericherry	2
18.	18.	VG	Flower: pubescence of style		
(+)			absent	Campbell 1327	1
QL			present	Saint-Pierre	9
19.	19. (*)	VG	Peduncle: abscission layer		
(+)			absent	Aledo, Bandera, Count, Lerica	1
QL G			present	Montfavet H 63.5, Roma	9
20.	20. (*)	VG/MS	Only for varieties with peduncle abscission layer present: Peduncle: length		
(+)			short	Cerise, Ferline, Montfavet H 63.18, Rossol	3
QN			medium	Dario, Primosol	5
			long	Erlidor, Ramy, Ranco	7
21.	21. (*)	VG	Fruit: green shoulder (before maturity)		
(+)		(b)	absent	Felicia, Rio Grande, Trust	1
QL G			present	Daniela, Montfavet H 63.5	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
22.	22.	VG	Only for varieties with a green shoulder: Fruit: extent of green shoulder (before maturity)		
(+)		(b)	very small	Daniela	1
QN			small	Ballet, Cristy, Firestone, Siluet	3
			medium	Erlidor, Foxy, Montfavet H 63.5	5
			large	Cobra, Delisa, Epona, Manific	7
23.	23.	VG	Only for varieties with a green shoulder: Fruit: intensity of green colour of shoulder (before maturity)		
(+)		(b)	light	Ballet, Daniela, Juboline	3
QN			medium	Montfavet H 63.5, Siluet	5
			dark	Ayala, Erlidor, Xenon	7
24.	24.	VG	Fruit: intensity of green colour excluding shoulder (before maturity)		
(+)		(b)	very light	Clarée	1
QN			light	Capello, Daniela, Duranto, Durinta, Trust	3
			medium	Marmande, Rody	5
			dark	Ayala, Centella, Tatiana, Uragano	7
			very dark	Verdi	9
25.	25 (*).	VG	Fruit: green stripes (before maturity)		
(+)		(b)	absent	Daniela	1
QL G			present	Green Zebra, Tigerella	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
26.	26.	VG	Fruit: size		
	(*)	(c)	very small	Cerise, Sweet 100	1
QN			small	Early Mech, Europeel, Roma	3
			medium	Alphamech, Diego	5
			large	Carmello, Ringo	7
G			very large	Erlidor, Lydia, Muril	9
27.	27.	VG/MS	Fruit: ratio length/diameter		
	(*)	(c)	very small (very compressed)	Campbell 28, Marmande VR	1
QN			small (moderately compressed)	Alicia	3
			medium	Early Mech, Peto Gro	5
			large (moderately elongated)	Rimone, Rio Grande	7
			very large (very elongated)	Elko, Macero II	9
28.	28.	VG	Fruit: shape in longitudinal section		
	(*)	(c)	flattened	Campbell 28, Marmande VR	1
(+)			oblate	Montfavet H 63.4, Montfavet H 63.5	2
PQ			circular	Cerise, Moneymaker	3
			oblong	Early Mech, Peto Gro	4
			cylindric	Hypeel 244, Macero II, San Marzano 2	5
			elliptic	Alcaria, Castone	6
			cordate	Valenciano	7
			ovate	Dualrow, Soto	8
			obovate	Duquesa, Estelle, Rimone, Rio Grande	9
			pyriform	Europeel	10
G			obcordate	Cuero de Ponente, Magno	11

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
29.	29.	VG	Fruit: ribbing at peduncle end		
	(*)	(c)	absent or very weak	Calimero, Cerise	1
(+)			weak	Early Mech, Hypeel 244, Melody, Peto Gro, Rio Grande	3
QN			medium	Montfavet H 63.4, Montfavet H 63.5	5
			strong	Campbell 1327, Carmello, Count	7
			very strong	Costoluto Fiorentino, Ingrid, Marmande VR	9
30.	30.	VG	Fruit: depression at peduncle end		
(+)		(c)	absent or very weak	Europeel, Heinz 1706, Rossol, Sweet Baby	1
QN			weak	Futuria, Melody	3
			medium	Carmello, Count, Fandango, Saint-Pierre	5
			strong	Ballon Rouge, Marmande VR	7
31.	31.	VG/MS	Fruit: size of peduncle scar		
(+)		(c)	very small	Cerise, Heinz 1706, Sweet Baby	1
QN			small	Early Mech, Peto Gro, Rio Grande	3
			medium	Montfavet H 63 4, Montfavet H 63 5	5
			large	Apla, Campbell 1327, Carmello, Fandango, Flora Dade	7
			very large	Marmande VR	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
32.	32.	VG/MS	Fruit: size of blossom scar		
(+)		(c)	very small	Cerise, Early Mech, Europeel, Heinz 1706, Peto Gro, Rio Grande	1
QN			small	Montfavet H 63.4, Montfavet H 63.5	3
			medium	Alphamech, Apla, Carmello, Floradade	5
			large	Campbell 1327, Count, Marmande VR, Saint-Pierre	7
			very large	Rozova Magia	9
33.	33.	VG	Fruit: shape at blossom end		
(+)		(c)	indented	Marmande VR, Super Mech	1
QN			indented to flat		2
			flat	Montfavet H 63.4, Montfavet H 63.5	3
			flat to pointed	Cal J, Early Mech, Peto Gro	4
			pointed	Europeel, Heinz 1706, Hypeel 244, Roma VF	5
34.	34.	VG/MS	Fruit: diameter of core in cross section in relation to total diameter		
(+)		(c)	very small	Cerise	1
QN			small	Early Mech, Europeel, Heinz 1706, Peto Gro, Rio Grande, Rossol	3
			medium	Montfavet H 63.4, Monfavet H 63.5	5
			large	Apla, Campbell 1327, Carmello, Count, Fandango, Floradade	7
			very large	Marmande VR, Valenciano	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
35.	35.	VG	Fruit: thickness of pericarp		
(+)		(c)	very thin	Cerise	1
QN			thin	Marmande VR	3
			medium	Carmello, Europeel, Floradade, Heinz 1706, Montfavet H 63.5	5
			thick	Cal J, Daniela, Ferline, Peto Gro, Rio Grande	7
			very thick	Myriade, Rondex	9
36.	36.	VG/MS	Fruit: number of locules		
	(*)	(c)	only two	Early Mech, Europeel, San Marzano	1
(+)			two and three	Alphamech, Futuria	2
QN			three and four	Montfavet H 63.5	3
			four, five or six	Raïssa, Tradiro	4
G			more than six	Marmande VR	5
37.	37.	VG	Fruit: colour at maturity		
	(*)	(c)	cream	Jazon, White Mirabell	1
(+)			yellow	Goldene Königin, Yellow Pear	2
PQ			orange	Sungold	3
			pink	Aichi First	4
			red	Daniela, Ferline, Montfavet H 63.5	5
			brown	Ozyrys	6
G			green	Green Grape, Green Zebra	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
38.	38.	VG	Fruit: colour of flesh (at maturity)		
	(*)	(c)	cream	Jazon	1
(+)			yellow	Jubilée	2
PQ			orange	Sungold	3
			pink	Regina	4
			red	Ferline, Saint-Pierre	5
			brown	Ozyrys	6
			green	Green Grape, Green Zebra	7
39.	39.	VG	Fruit: glossiness of skin		
QN		(c)	weak	Josefina	1
			medium	Roncardo	2
			strong	Mecano	3
40.	41.	VG	Fruit: firmness		
	(*)	(c)	very soft	Marmande VR	1
(+)			soft	Trend	3
QN			medium	Cristina	5
			firm	Fernova, Konsul, Tradiro	7
			very firm	Daniela, Karat, Lolek	9
41.	43.	MS	Time of flowering		
(+)			early	Feria, Primabel	3
QN			medium	Montfavet H 63.5, Prisca	5
			late	Manific, Saint-Pierre	7
42.	44.	MG	Time of maturity		
	(*)		very early	Dolcevita, Sungold, Sweet Baby	1
(+)			early	Bianca, Rossol, Shiren	3
QN			medium	Gourmet, UC 82B	5
			late	Arletta, Durinta	7
			very late	Daniela	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
43. (*)	46. (*)	VG	Resistance to <i>Meloidogyne</i> incognita (Mi)		
(+)			susceptible	Casaque Rouge	1
QN			moderately resistant	Campeon	2
G			highly resistant	Anabel, Anahu	3
44. (*)	47. (*)	VG	Resistance to <i>Verticillium</i> sp. (Va and Vd) - Race 0		
(+)			absent	Anabel, Marmande verte	1
QL G			present	Dianela, Marmande VR	9
45. (+)	48.	VG	Resistance to <i>Fusarium</i> oxysporum f. sp. lycopersici (Fol)		
45.1 (*)	48.1 (*)		- Race 0 (ex 1)		
QL			absent	Marmande verte	1
G			present	Anabel, Marporum, Marsol	9
45.2 (*)	48.2 (*)	VG	- Race 1 (ex 2)		
QL			absent	Marmande verte	1
G			present	Motelle, Walter	9
45.3	48.3	VG	- Race 2 (ex 3)		
QL			absent	Marmande verte, Motelle	1
			present	Alliance, Florida, Ivanohé, Tributes	9
46.	49.	VG	Resistance to <i>Fusarium</i> oxysporum f. sp. radicis- lycopersici (Forl)		
(+)			absent	Motelle	1
QL			present	Momor	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
47. (+)	50. (+)	VG	Resistance to Fulvia fulva (Ff) (ex Cladosporium fulvum)		
47.1	50.1		- Race 0		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine, Sonato, Vemone	9
47.2	50.2	VG	- Group A		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine, Sonato	9
47.3	50.3	VG	- Group B		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine, Sonato, Vemone	9
47.4	50.4	VG	- Group C		
QL			absent	Monalbo	1
			present	Angela, Estrella, Sonatine	9
47.5	50.5	VG	- Group D		
QL			absent	Monalbo	1
			present	Estrella, Sonatine, Vemone	9
47.6	50.6	VG	- Group E		
QL			absent	Monalbo	1
			present	Sonatine	9
48. (+)	51. (+)		Resistance to Tomato mosaic virus (ToMV)		
48.1 (*)	51.1	VG	- Strain 0		
QL			absent	Monalbo	1
G			present	Mobaci, Mocimor, Moperou	9
48.2	51.2	VG	- Strain 1		
QL	QL		absent	Monalbo	1
			present	Mocimor, Moperou	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
48.3	51.3	VG	- Strain 2		
	QL		absent	Monalbo	1
			present	Mobaci, Mocimor	9
49.	52.	VG	Resistance to <i>Phytophthora infestans</i> (Pi)		
(+)			absent	Heinz 1706, Saint Pierre	1
QL			present	Fline, Heline, Pieraline, Pyros	9
50.	53.	VG	Resistance to <i>Pyrenochaeta lycopersici</i> (PI)		
(+)	(+)		absent	Montfavet H 63.5	1
QL	QL		present	Kyndia, Moboglan, Pyrella	9
51.	54.	VG	Resistance to Stemphylium spp.		
(+)			absent	Monalbo	1
QL			present	Motelle	9
52.	55.	VG	Resistance to <i>Pseudomonas</i> syringae pv. tomato (Pst)		
(+)			absent	Monalbo	1
QL			present	Ontario 7710	9
53.	56.	56. VG Resistance to <i>Ralstonia</i> solanacearum (Rs) - Race 1			
(+)			absent	Floradel	1
QL			present	Caraïbo	9
54.	57.	VG	Resistance to Tomato yellow leaf curl virus (TYLCV)		
(+)			absent	Montfavet H 63.5	1
QL			present	Anastasia, Mohawk, TY 20	9
55.	58.	VG	Resistance to Tomato spotted wilt virus (TSWV)  — Race 0		
(+)			absent	Montfavet H 63.5	1
QL G			present	Lisboa	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
56.	59.	VG	Resistance to <i>Leveillula taurica</i> (Lt)		
(+)			absent	Montfavet H 63.5	1
QL			present	Atlanta	9
57.	60.	VG	Resistance to <i>Oidium</i> neolycopersici (On) (ex <i>Oidium</i> lycopersicum (OI))		
(+)			absent	Montfavet H 63.5	1
QL			present	Romiro	9
58.	61.	VG	Resistance to Tomato torrado virus (ToTv)		
(+)			absent	Daniela	1
QL			present	Matias	9

#### 8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

#### 8.1 Explanations covering several characteristics

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

- (a) In the case of indeterminate varieties, observations on the plant, stem and leaf should be done after a fruit set on at least five trusses and before ripening of the second truss. In the case of determinate varieties, all observations on the plant and leaves should be done after a fruit set on the second truss. Observations should be done before deterioration of the leaves.
- (b) Observations should be made on the plant before maturity (see Ad. 44).
- (c) Observations should be made on fruits at maturity (see Ad. 44) from the second or higher truss, avoiding first and last mature fruits on truss.

#### 8.2 Explanations for individual characteristics

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





#### Ad. 2: Plant: growth type

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

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

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

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

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

#### Ad. 4. Stem: anthocyanin coloration of upper third

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

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

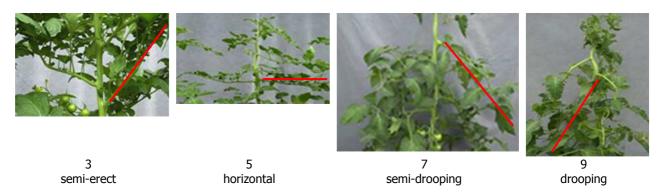
The length of the internode should be observed/measured at one time for the whole trial, e.g. after a fruit set on approximately 5 nodes. The total length of the stem should be observed/measured between the  $1^{st}$  and  $4^{th}$  trusses. In case of measurements, this measure is divided by the number of internodes in between, an indication of the length of the internode is given.

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

The height of the plant should be measured at one time for the whole trial, e.g. 60 days after planting, or after a fruit set on approximately 5 nodes, or when the first variety in the trial has reached the wire in the green house or the top of the stake.

#### Ad. 7: Leaf: attitude

The attitude of the middle third part of the leaves in respect to the main stem should be observed.



## Ad. 10: Leaf: type of blade

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



1 pinnate



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

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

## Ad. 13: Leaf: glossiness

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

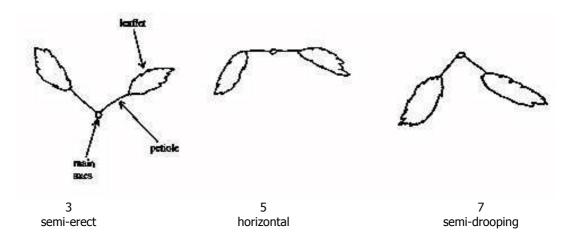
## Ad. 14: Leaf: blistering

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





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



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

## Ad. 16: Inflorescence: type

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



uniparous

multiparous (biparous)

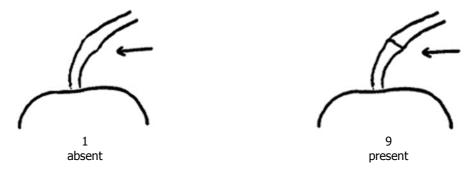


multiparous (triparous)

## Ad. 18: Flower: pubescence of style

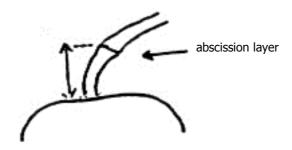
Some varieties with pubescence of style "present" may have only rare and small hairs at the base of the style.

## Ad. 19: Peduncle abscission layer



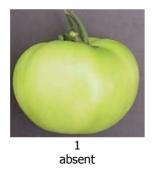
Varieties which have only a collar instead of an abscission layer are heterozygous for the gene which controls the presence of the joint. These varieties are considered jointless and the abscission layer is considered absent.

## Ad. 20: Only varieties with peduncle abscission layer present: Peduncle: length



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

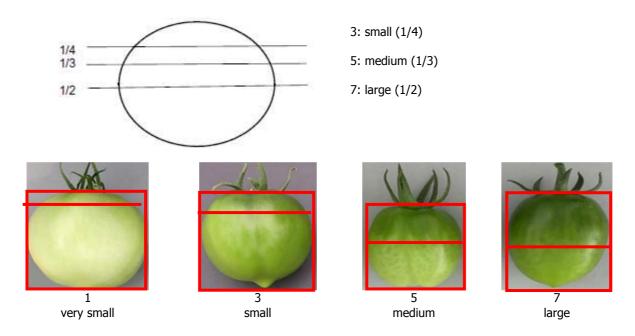
The gene for green shoulder might not be clearly expressed in some conditions, which is why it is important to have the example variety 'Daniela' to observe the expression of these characteristics.





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

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



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

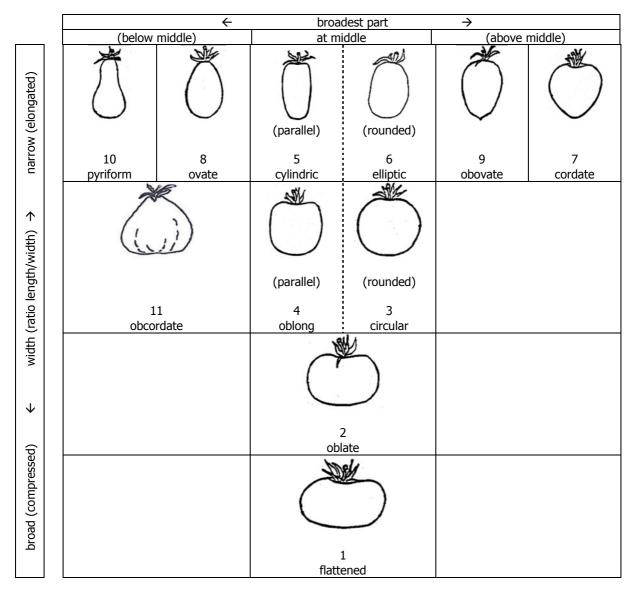
Intensity of green colour of shoulder and intensity of green colour excluding shoulder have to be observed on the same scale. This means that the note for intensity of green colour of shoulder should be higher than the note for intensity of green colour excluding shoulder, or in exceptional cases the same if the difference in intensity is very small. The gene for green shoulder might not be clearly expressed in some conditions, which is why it is important to have the example variety 'Daniela' to observe the expression of these characteristics.

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

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



Ad. 28: Fruit: shape in longitudinal section



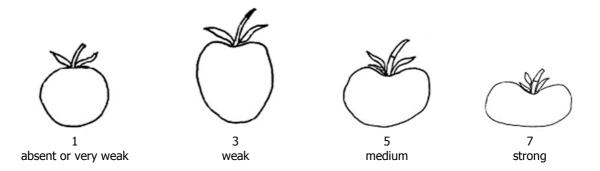
The apex is considered to be the part that is farthest from the peduncle end.

## Ad. 29: Fruit: ribbing at peduncle end

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



## Ad. 30: Fruit: depression at peduncle end



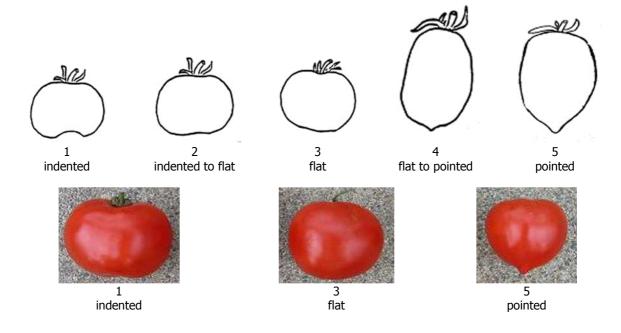
## Ad. 31: Fruit: size of peduncle scar

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

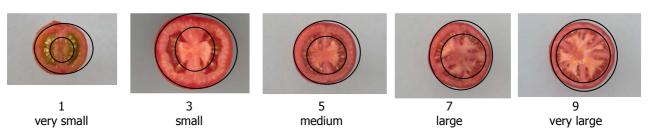
## Ad. 32: Fruit: size of blossom scar

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

#### Ad. 33: Fruit: shape at blossom end



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

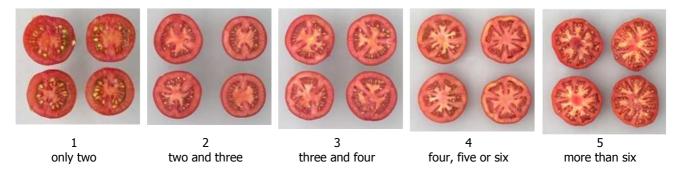


#### Ad. 35: Fruit: thickness of pericarp

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

## Ad. 36: Fruit: number of locules

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



#### Ad. 37: Fruit: colour (at maturity)

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

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

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

The colour at maturity has to be observed at maturity (see Ad. 44).

## Ad. 40: Fruit: firmness

## Method

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

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

#### Ad. 41: Time of flowering

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

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

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

## Ad. 42: Time of maturity

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

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

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

1. Pathogen	Meloidogyne incognita
3. Host species	Solanum lycopersicum
4. Source of inoculum	Naktuinbouw (NL¹) or GEVES² (F)
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	
8.3 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	Not applicable
9.3 Control varieties	
Susceptible:	Clairvil, Casaque Rouge
Moderately resistant:	Campeon
Highly resistant:	Anahu, Anabel, Anahu x Monalbo
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
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
11.4 Off-types	resistant varieties may have a few plants with a few galls
12. Interpretation of data in terms of	
absent (susceptible)	[1] growth strongly reduced, high gall count
intermediate (moderately resistant	
present (highly resistant)	[3] present; no growth reduction, no galls
13. Critical control points:	and a second based above of the Salaman
Avoid rotting of roots; high temperatu	re causes preakdown of resistance

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

<sup>&</sup>lt;sup>2</sup> GEVES; Valerie.GRIMAULT@geves.fr

<sup>32</sup> 

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

1. Pathogen	Verticillium dahliae (see note below)
3. Host species	Solanum lycopersicum
4. Source of inoculum	Naktuinbouw <sup>3</sup> (NL) and GEVES <sup>4</sup> ( <i>F</i> )
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 inoculumsp	
8.8 Shelf life/viability inoculums1	
9. Format of the test	,
9.1 Number of plants per genotype	35 seed for 24 plants
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible	Flix, Marmande verte, Clarion, Santonio, Anabel
	Monalbo, Elias, Monalbo x Marmande verte, Daniela, Marmande VR
9.4 Test design	20 plants inoculated, 2 blanks at least
9.5 Test facility	greenhouse or climate room
9.6 Temperature	optimal 20-25°C, 20-22°C after inoculation
9.7 Light	12 h or longer
10. Inoculation	<b>.</b>
10.1 Preparation inoculums	aerated, liquid culture (8.4)
10.2 Quantification inoculums	count spores, adjust to 106 per ml
10.3 Plant stage at inoculation	cotyledon to 3rd leaf
10.4 Inoculation method	roots are immersed for 4 to 15 min in spore suspension.
10.5 Final observations	33 days after inoculation
11. Observations	
11.1 Method	visual
11.2 Observation scale	growth retardation, wilting, chlorosis, and vessel browning
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant
i	and susceptible controls
12. Interpretation of data in terms of	
absent [1	
present [9	
13. Critical control points	• •

All symptoms may be present in resistant varieties, but the severity will be distinctly less than in susceptible varieties. Usually resistant varieties will show significantly less growth retardation then susceptible varieties. Observation of vessel browning is important for diagnosis. Usually, vessel browning will not extend to the 1<sup>st</sup> leaf in resistant varieties. Many hybrid varieties are heterozygous and appear to have mild symptoms in the biotest.

Note: Resistance to V. dahliae based in the Ve gene is also effective to V. albo-atrum. Isolates of both fungal species may be used to evaluate the UPOV characteristic "Resistance to V. dahliae" or V. albo-atrum as long as the isolate belongs to the non-Ve breaking race 0. Resistance-breaking isolates have been described in both species.

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

Pathogen	Solanum lycopersicum
<ul><li>6. Establishment isolate identity</li><li>7. Establishment pathogenicity</li></ul>	Individual strains may vary in pathogenicity use differential varieties (see 9.3) on tomato varieties

<sup>&</sup>lt;sup>3</sup> Naktuinbouw; resistentie@naktuinbouw.nl

<sup>&</sup>lt;sup>4</sup> GEVES; Valerie.GRIMAULT@geves.fr

O Multipliantian in a culum	
Multiplication inoculum     Multiplication medium	Potato Dextrose Agar, Medium "S" of Messiaen
8.4 Inoculation medium	water for scraping agar plates or Czapek-Dox culture medium (7 day-old
o. i mocdiation mediam	aerated culture)
8.6 Harvest of inoculum	filter through double muslin cloth
8.7 Check of harvested inoculum	spore count; adjust to 106 per ml
8.8 Shelf-life/viability inoculum	4-8 h, keep cool to prevent spore germination
9. Format of the test	. o ty keep ees to provent spere germmation
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates	Not applicable
9.3 Control varieties for the test with	
Susceptible	Marmande, Marmande verte, Resal
Resistant for race 0 only	Marporum, Larissa, "Marporum x Marmande verte", Marsol
Resistant for race 0 and 1	Motelle, Gourmet, Mohawk
Control varieties for the test with	
Susceptible	Marmande verte, Cherry Belle, Roma
Resistant for race 0 only	Marporum, Ranco
Resistant for race 0 and 1	Tradiro, Odisea
Remark:	Ranco is slightly less resistant than Tradiro
Control varieties for the test with	
Susceptible for race 0, 1 and 2.	Marmande verte, Motelle, Marporum
Resistant for race 0, 1 and 2	Tributes, Murdoch, Marmande verte x Florida
9.4 Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility	glasshouse or climate room 24-28°C (severe test, with mild isolate)
9.6 Temperature	20-24°C (severe test, with mild 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	signary acidic peaceson is opamaly reception marine sactarola mater saces
10.1 Preparation inoculums	Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or
·	scraping of plates
10.2 Quantification inoculums	spore count, adjust to 106 spores 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 for 5-15 min;
	trimming of roots is an option
10.7 Final observations	14-21 days after inoculation
11. Observations	- Arrest
11.1 Method	visual
11.2 Observation scale	Symptoms: growth retardation, wilting, yellowing, vessel browning
11.3 Validation of test	extending above cotyledon evaluation of variety resistance should be calibrated with results of
11.5 Validation of test	resistant and susceptible controls
12. Interpretation of data in terms of	
absent	[1] severe symptoms
present	[9] mild or no symptoms
13. Critical control points	F-3 vol. 110 e/111/prome
	lum pressure due to differences in isolate, spore concentration, soil humidity
	derline R/S help to compare between labs.

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

1. Pathogen	Fusarium oxysporum f. sp. radicis-lycopersici
Host species      Source of inoculum	Solanum lycopersicum Naktuinbouw <sup>7</sup> (NL) and GEVES <sup>8</sup> ( <i>F</i> )
Isolate      Establishment pathogenicity	symptoms on susceptible tomato
Multiplication inoculum     Multiplication medium	Potato Dextrose Agar, or Medium agar "S" of Messiaen
8.4 Inoculation medium 8.6 Harvest of inoculum	Water for scraping agar plates or Czapek-Dox (7 d-old aerated culture) filter through double muslin cloth
8.7 Check of harvested inoculum	spore count; adjust to 10 <sup>6</sup> per ml
<ul><li>8.8 Shelf life/viability inoculum</li><li>9. Format of the test</li></ul>	4-8 h, keep cool to prevent spore germination
9.1 Number of plants per genotype	at least 20
<ul><li>9.2 Number of replicates</li><li>9.3 Control varieties</li></ul>	Not applicable
Susceptible:	Motelle, Moneymaker
Resistant:	Momor, "Momor x Motelle"
Remark:	"Momor x Motelle" has slightly weaker resistance than Momor
9.4 Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility	glasshouse or climate room
9.6 Temperature	24-28°C (severe test, with mild isolate) 17-24°C (mild test, with severe isolate)
9.7 Light	at least 12 hours per day
9.8 Season	all seasons
9.9 Special measures	slightly acidic peat soil is optimal;
	keep soil humid but avoid water stress
10. Inoculation	
10.1 Preparation inoculum	aerated culture or scraping of plates
10.2 Quantification inoculum	spore count, adjust to 10 <sup>6</sup> spores per ml
10.3 Plant stage at inoculation 10.4 Inoculation method	12-18 d, cotyledon to third leaf roots and hypocotyls are immersed in spore suspension
10.4 Inoculation metriod	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
44 2 Wellderland of back	Root degradation Necrotic pinpoints and necrotic lesions on stems
11.3 Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of data in terms of	
<u>absent</u> [1	
<u>present</u> [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

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## Ad. 47.1 - 47.7: Resistance to Fulvia fulva (Ff) (ex Cladosporium fulvum)

1. Pathogen	Fulvia fulva (ex Cladosporium fulvum)
3. Host species	Solanum lycopersicum
4. Source of inoculum	Naktuinbouw <sup>9</sup> (NL) 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 nathogenicity	symptoms on susceptible tomato

8.1 Multiplication medium		
8.8 Shelf life/viability inoculum		
9. Format of the test 9.1 Number of plants per genotype 9.2 Number of replicates		
9.1 Number of palnts per genotype 9.2 Number of replicates	• •	4 hours, keep cool
9.2 Number of replicates		
9.3 Control varieties Susceptible:		
Susceptible:		Not applicable
Resistant for race 0:		Manalla Manayanalay
Nagabond × IVT 1149, IVT 1154  Resistant for race group A:		
Resistant for race group A:	Resistant for race u:	
Resistant for race group B:	Resistant for race group A:	
Resistant for race group C:		
Resistant for race group D: Resistant for race group E:  Sonatine, Jadviga, Rhianna, IVT 1154 glasshouse or climate room day: 22° C, night: 20° or day: 25° C, night 20° C 12 hours or longer depending on facility and weather, there may be a need to raise the humidity, e.g. humidity tent closed 3-4 days after inoculation and after this, 66% until 80% closed during day, until end  10. Inoculation 10.1 Preparation inoculum		
Resistant for race group E:		
9.5 Test facility		
9.7 Light		
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  10.1 Preparation inoculum	9.6 Temperature	day: 22° C, night: 20°or day: 25°C, night 20°C
humidity, e.g. humidity tent closed 3-4 days after inoculation and after this, 66% until 80% closed during day, until end  10. Inoculation  10.1 Preparation inoculum	9.7 Light	12 hours or longer
this, 66% until 80% closed during day, until end  10. Inoculation  10.1 Preparation inoculum	9.9 Special measures	
10. Inoculation 10.1 Preparation inoculum		
prepare evenly colonized plates, e.g. 1 for 36 plants; remove spores from plate by scraping with water with Tween20; filter through double muslin cloth  10.2 Quantification inoculum		this, 66% until 80% closed during day, until end
remove spores from plate by scraping with water with Tween20; filter through double muslin cloth  10.2 Quantification inoculum		
filter through double muslin cloth  10.2 Quantification inoculum	10.1 Preparation inoculum	
10.2 Quantification inoculum		
10.3 Plant stage at inoculation	10.2.0	
10.4 Inoculation method		
10.7 Final observations		
11. Observations  11.1 Method		
11.1 Method		14 days after moculation
11.2 Observation scale		visual inspection of abavial side of inoculated leaves
11.3 Validation of test		
resistant and susceptible controls  11.4 Off-types		
11.4 Off-types	11.5 Validation of test illimines	
12. Interpretation of data in terms of UPOV characteristic states     absent [1] symptoms     present [9] no symptoms  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.	11.4 Off-types	
absent [1] symptoms present [9] no symptoms  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.		
present [9] no symptoms  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.	•	
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.		
Fungal plates will gradually become sterile after 6-10 weeks. Store good culture at -80°C.		
For practical purposes, it is not possible to keep plants longer than 14 days inside a tent.		
	For practical purposes, it is not possible	e to keep plants longer than 14 days inside a tent.

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## Ad.481.1 – 48.3: Resistance to Tomato mosaic virus (ToMV) - Strains 0, 1 and 2

1. Pathogen	Tomato mosaic virus
3. Host species	Solanum lycopersicum
4. Source of inoculum	Naktuinbouw <sup>11</sup> (NL) or GEVES <sup>12</sup> (F)
5. Isolate	Strain 0 <sub>7</sub> (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 Nicotiana tabacum "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum	fresh>1 day, desiccated>1year
9. Format of the test	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates	Not applicable

9.3 Control varieties

Susceptible ..... Marmande, Monalbo

Resistant for ToMV: 0 and 2 ..... Mobaci Resistant for ToMV: 0 and 1 .... Moperou

Resistant with necrosis..... "Monalbo x Momor"

Resistant ..... Gourmet

blank treatment with PBS and carborundum or similar buffer 9.4 Test design.....

9.5 Test facility..... Glasshouse or climate room

9.6 Temperature..... 24 to 26°C

9.7 Light ..... 12 hours or longer

9.8 Season ..... symptoms are more pronounced in summer

10. Inoculation

10.1 Preparation inoculum ...... 1 g leaf with symptoms with 10 ml PBS or similar buffer

Homogenize, add carborundum to buffer (1 g/30ml)

10.6 Plant stage at inoculation ...... cotyledons or 2 leaves

10.4 Inoculation method ..... gentle rubbing

10.7 Final observations..... 11-21 days after inoculation

11. Observations

11.1 Method.....

11.2 Observation scale ..... Symptoms of susceptibility:

Mosaic in top, <u>leaf</u> malformation

Symptoms of resistance (based on hypersensitivity):

Local Necrosis, Top necrosis, Systemic Necrosis 11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of

resistant and susceptible controls

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

12. Interpretation of data in terms of UPOV characteristic states

symptoms of susceptibility absent [1]

present Ī9Ī no symptoms, or symptoms of hypersensitive resistance

### 13. Critical control points:

Temperature and light may influence the development of necrosis. More light means more necrosis. At temperatures above 26°C the resistance may break down.

Resistant heterozygous varieties may have symptomless plants and plants with severe necrosis; in spite of apparent segregation the sample may be evaluated as uniform for resistance.

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

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

1. Pathogen ..... Phytophthora infestans 3. Host species ..... Solanum lycopersicum

4. Source of inoculum .....

5. Isolate ..... highly pathogenic on tomato

6. Establishment isolate identity ..... 7. Establishment pathogenicity...... biotest

8. Multiplication inoculum

8.1 Multiplication medium..... V8 Agar or PDA or Malt Agar medium

8.2 Multiplication variety..... susceptible tomato variety

8.3 Plant stage at inoculation...... 4 weeks 8.4 Inoculation medium..... water 8.5 Inoculation method ..... spraying

8.6 Harvest of inoculum ..... wash spores from wetted plates

8.7 Check of harvested inoculum.... count sporangiospores 8.8 Shelf life/viability inoculum...... 4 h after chilling at 8-10°C

9. Format of the test

9.1 Number of plants per genotype 20

9.2 Number of replicates...... Not applicable

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9.3 Control varieties Susceptible ..... Saint Pierre, Heinz 1706 Resistant ..... Pieraline, Heline, Pyros, "Pieraline x Pieralbo", Fline Remark: ..... heterozygote varieties may have a slightly lower level of expression of resistance. 9.5 Test facility..... glasshouse 18°C 9.6 Temperature..... 9.7 Light ..... after inoculation darkness during 24 hours, thereafter 10 hour darkness per 24 hours 9.9 Special measures ..... humidity tent during four days after inoculation 10. Inoculation wash spores from sporulating leaves, chill at 8-10°C 10.1 Preparation inoculum ..... Chilling will induce zoospore release Use fresh spores from repeated infection cycles on tomato plants during 3 Remark..... weeks before inoculation 10.2 Quantification inoculum ....... count sporangiospores; adjust to 104 spores per ml 10.3 Plant stage at inoculation ...... 10 leaves developed (6 to 7 weeks) 10.4 Inoculation method ..... spraving 10.7 Final observations..... 5-7 days after inoculation 11. Observations 11.1 Method..... 11.2 Observation scale ..... Symptoms: water-soaked lesions, yellowing, and death 11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls 12. Interpretation of data in terms of UPOV characteristic states absent [1] severe symptoms present [9] no or mild symptoms resistance is only well-expressed in the adult plant 13. Critical control points:.....

#### Ad. 50: Resistance to Pyrenochaeta lycopersici (PI)

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 <u>lose</u> its pathogenicity within a
	week after isolation on an agar medium
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	Not applicable
9.3 Control varieties	
susceptible:	Montfavet H 63.5
resistant:	Kyndia, Moboglan, Pyrella
9.5 Test facility	greenhouse or climate cell
9.6 Temperature	day 24°C, night 14°C
9.7 Light	12 h minimum
10. Inoculation	
10.1 Preparation inoculum	e.g. double-autoclaved mixture of soil with 10% oatmeal added
10.2 Dlank skape at incordation	e.g. Incubate for 10-14 d at 20°C with occasional, repeated turning
10.2 Plant stage at inoculation	6 weeks
10.3 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 evaluation of variety resistance should be calibrated with results of 11.3 Validation of test ..... resistant and susceptible controls 12. Interpretation of data in terms of UPOV characteristic states absent [1] symptoms present [9] no symptoms 13. Critical control points: The fungus loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants. Ad. 51: Resistance to Stemphylium spp. 1. Pathogen ..... Stemphylium spp. e.g. Stemphylium solani (see note below) 3. Host species ...... Solanum lycopersicum 4. Source of inoculum ..... GEVES (Fr) 5. Isolate ..... 7. Establishment pathogenicity...... biotest 8. Multiplication inoculum 8.1 Multiplication medium..... PDA (12 hours per day under near-ultraviolet light to induce sporulation) or V8 9. Format of the test 9.1 Number of plants per genotype 20 at least 9.2 Number of replicates ..... Not applicable 9.3 Control varieties Susceptible: ..... Resistant: ..... Motelle, F1 Motelle x Monalbo 9.5 Test facility..... greenhouse or climate cell 9.6 Temperature..... 24°C 9.7 Light ..... 12 hours minimum 9.9 Special measures ..... incubation in tunnel with 100 % relative humidity or humidity tent closed 5 days after inoculation, after this, 80% until end 10. Inoculation 10.1 Preparation inoculum..... sporulating plates (8.1) are scraped and air-dried overnight. The next day plates are soaked and stirred for 30 min in a beaker with demineralized water, or sporulating plates are scraped with water with Twee. The spore suspension is sieved through a double layer of muslin.  $5.10^3 - 10^5$  spores per ml 10.2 Quantification inoculum ....... 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; vellowing of leaves 11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls 12. Interpretation of data in terms of UPOV characteristic states absent [1] symptoms (11.2) present [9] no symptoms, or less than resistant standard 13. Critical control points:..... 8.1 and 10.1 Note: Some isolates of Stemphylium cannot be classified easily as either Stemphylium solani or a related species. These Stemphylium isolates may still be useful for identifying resistance to Stemphylium solani. Ad. 52: Resistance to *Pseudomonas syringae* pv. Tomato (Pst) Pseudomonas syringae pv. tomato (see note below) 1. Pathogen

Solanum lycopersicum

GEVES<sup>13</sup> (FR) or Naktuinbouw<sup>14</sup> (NL)

3. Host species .....

4. Source of inoculum .....

5. Isolate .....

6. Establishment isolate identity 7. Establishment pathogenicity 8. Multiplication inoculum 8.1 Multiplication medium 8.2 Multiplication variety 8.4 Inoculation medium 8.8 Shelf life/viability inoculum 9. Format of the test	biotest  King's B agar medium, darkness Susceptible variety water plates become old after 10 days
9.1 Number of plants per genotype	20 at least
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible:	Monalbo
Resistant:	Ontario 7710, "Monalbo x Ontario 7710", Tradiro, Hypeel 45
9.5 Test facility	greenhouse or growth chamber
9.6 Temperature	day: 22° C, night: 16° C or 20°C
9.7 Light	12 hours
9.9 Special measures	humidity tent needed for 3 days or longer
10. Inoculation	
10.1 Preparation inoculum	wash off spores from plate. Plate should be less than 2-4 days old.
10.2 Quantification inoculum	dilution plating, density 10 <sup>6</sup> colony forming units per ml
10.3 Plant stage at inoculation	three leaves expanded (20-22 days)
10.4 Inoculation method	spraying a bacterial suspension on leaves
10.7 Final observations	8 days after inoculation or longer
11. Observations	. devel
11.1 Method	visual
11.2 Observation scale	bacterial speck, greasy in appearance with marginal chlorosis pinpoint lesions < 1.0 mm
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of
	resistant and susceptible controls
12. Interpretation of data in terms of L	JPOV characteristic states
absent [1]	
present [9]	no symptoms or pinpoint lesions
13. Critical control points:	Strains may lose virulence in storage
·	·
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### Ad. 53: Resistance to Ralstonia solanacearum, (ex. Pseudomonas solanacearum) (Rs) - Race 1

Pathogen      Quarantine status      Host species      Source of inoculum	Ralstonia solanacearum (ex Pseudomonas solanacearum) yes (see note below) Solanum lycopersicum
5. Isolate	Race 1 has a wide host range, including tomato.
	Race 3 has a narrow host range, also including tomato
8. Multiplication inoculum	
8.1 Multiplication medium	Yeast Peptone Glucose (YPG) Agar or PYDAC
Special conditions:	25-30°C (Race 3 usually needs 20-23°C)
8.5 Inoculation method	2 ml of inoculum placed at the foot of each plantlet prior to transplanting
8.8 Shelf life/viability inoculum 9. Format of the test	suspension in sterile distilled water at 15°C (<1 year)
	20
9.1 Number of plants per genotype 9.2 Number of replicates	Not applicable
9.3 Control varieties	Not applicable
Susceptible:	Floradel
Resistant:	Caraibo
9.5 Test facility	climate room
9.6 Temperature	day: 26-30° C; night: 25° C
9.7 Light	10 - 12 hours
9.9 Special measures	high humidity
10. Inoculation	7
10.2 Quantification inoculum 10.3 Plant stage at inoculation	density 10 <sup>'</sup> colony forming units per ml three to four well-developed leaves (3 weeks)

10.4 Inoculation method

10.7 Final observations..... 3 weeks after inoculation

In intermediate resistance varieties, bacteria could be present in the lower 11. Observations .....

part of the plant

evaluation of variety resistance should be calibrated with results of 11.3 Validation of test .....

resistant and susceptible controls

12. Interpretation of data in terms of UPOV characteristic states

symptoms absent [1]

present [9] no symptoms, or less than resistant standard

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

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

Tomato yellow leaf curl virus (see note below) 1. Pathogen .....

2. Quarantine status ..... ves

3. Host species ..... Solanum lycopersicum

4. Source of inoculum ..... 5. Isolate ..... 8. Multiplication inoculum

symptomatic leaves may be stored at -70°C 8.6 Harvest of inoculum .....

9. Format of the test 9.1 Number of plants per genotype 20

9.2 Number of replicates ..... Not applicable

9.3 Control varieties

Susceptible: ..... Montfavet H 63.5

Resistant: ..... TY 20, Anastasia, Mohawk 9.5 Test facility..... field with natural disease pressure 9.9 Special measures ..... prevent spread of white-flies

10. Inoculation

10.3 Plant stage at inoculation ...... 6-12 weeks (adult plants)

vector (Bemisia white-flies carrying TYLCV) 10.4 Inoculation method .....

10.7 Final observations..... 1-2 months after inoculation

11. Observations

11.1 Method.....

11.2 Observation scale ..... Symptoms: leaf yellowing and curling

evaluation of variety resistance should be calibrated with results of 11.3 Validation of test .....

resistant and susceptible controls

12. Interpretation of data in terms of UPOV characteristic states

absent severe symptoms [1] no or mild symptoms present

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 55: Resistance to Tomato spotted wilt virus (TSWV)

Tomato spotted wilt virus (see note below) 1. Pathogen .....

2. Quarantine status ..... yes (see note below) Solanum lycopersicum 3. Host species .....

Naktuinbouw 15(NL), GEVES (FR)16 4. Source of inoculum .....

5. Isolate ..... race 0, preferably a thrips-transmission deficient variant

7. Establishment pathogenicity......

8. Multiplication inoculum

6 Harvest of inoculum ..... symptomatic leaves may be stored at -70°C

9. Format of the test

9.1 Number of plants per genotype

9.2 Number of replicates ..... Not applicable

9.3 Control varieties

Susceptible ..... Monalbo, Momor, Montfavet H 63.5 Tsunami, Bodar, Mospomor, Lisboa Resistant ..... 9.5 Test facility..... glasshouse or climatic chamber

20°C 9.6 Temperature..... 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 mechanical, rubbing with suspension  $< 10^{\circ}$  C 10.4 Inoculation method carborundum on inoculum cotyledons, 10.5 Final observations..... 7--21 days after inoculation 11. Observations 11.1 Method..... visual 11.2 Observation scale ..... Symptoms: top mosaic, bronzing, various malformations, necrosis 11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls 12. Interpretation of data in terms of UPOV characteristic states

absent [1] symptoms present [9] no symptoms

13. Critical control points:

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

Note: Option for testing without using the pathogen

Resistance to TSWV:0 is often based on the resistance gene Sw-5. The presence of the resistance gene Sw-5 can be detected by molecular marker Sw-5b-LRR (Garland et al. 2005) or by a co-dominant SCAR marker (Dianese et al. 2010). This molecular test is validated to be used instead of the pathotest, as foreseen in UPOV document TC/38/17 Add. - CAJ/45/5 Add. Under Option 1(a). Each molecular marker should be applied to a minimum of twenty plants and validated with proper controls.

If the biolomolecular test is inconclusive, then the pathotest needs to be carried out

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

1. Pathogen	Leveillula taurica
3. Host species	Solanum lycopersicum
<ul><li>4. Source of inoculum</li><li>5. Isolate</li></ul>	no long term storage method is available
8.1 Multiplication medium	detached leaves of a susceptible host plant
9.1 Number of plants per genotype	20
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible	onalbo , Montfavet H 63.5
ResistantA	tlanta
10. Inoculation	
10.3 Plant stage at inoculation	adult plants
10.4 Inoculation method	natural infection, mainly by wind dispersal of spores
10.7 Final observations	before harvest
11. Observations	
11.1 Method	visual
11.2 Observation scale	Symptoms: Yellow chlorotic spots on upper side of leaves, mycelium on abaxial side of leaves
Remark: Check cleistothecia under m	icroscope to confirm presence of Leveillula and not another powdery mildew.
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of
	resistant and susceptible controls
12. Interpretation of data in terms of	UPOV characteristic states
absent [:	L] symptoms
present [9	no symptoms, or less than resistant standard

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

1. Pathogen 3. Host species 4. Source of inoculum 5. Isolate 7. Establishment pathogenicity 8. Multiplication inoculum 8.1 Multiplication medium	Oidium neolycopersici (Powdery mildew) Solanum lycopersicum - see remark under 13 biotest  plant
8.3 Plant stage at inoculation 8.4 Inoculation medium	24°C during the day; 18°C during the night water
8.5 Inoculation method	see 10.4
8.6 Harvest of inoculum	by washing off
8.7 Check of harvested inoculum	check for contaminants under microscope
8.8 Shelf-life/viability inoculum	1-2 hours
9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	Not applicable
9.3 Control varieties	Marrier Martiferent II 62 F
Susceptible:	Momor, Montfavet H 63.5
Resistant tomato:	Atlanta, Romiro, PI-247087
9.5 Test facility	glasshouse
9.6 Temperature	20°C or 18/24°C 12 hours
9.7 Light 10. Inoculation	12 Hours
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	7 To day's after inocalation
11.1 Method	visual
11.2 Observation scale	0. no sporulation
	necrotic points and sometimes locally restricted sporulation
	2. moderate sporulation
	3. abundant sporulation
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of
	resistant and susceptible controls
12. Interpretation of data in terms of	UPOV characteristic states
absent [1	
present [9	] No or restricted sporulation
13. Critical control points:	

Resistance-breaking isolates should be avoided. Resistance to O. neolycopersici is usually race-specific. However, as long as a differential series of tomato genotypes with well-defined resistances is lacking, it will remain hard to conclude that different races of O. neolycopersici exist.

#### Ad. 58: Resistance to Tomato torrado virus (ToTv)

<ol> <li>Pathogen</li></ol>	Tomato Torrado Virus in regions with temperate climate Solanum lycopersicum
7. Establishment pathogenicity	biotest
8. Multiplication inoculum	5.0000
8.1 Multiplication medium	Nicotiana tabacum 'Xanthi'
8.3 Plant stage at inoculation	cotyledon to first leaf
8.5 Inoculation method	see 10.4
8.6 Harvest of inoculum	after 3 weeks
8.7 Check of harvested inoculum	plants yellow, systemic infection
8 8 Shelf-life/viability inoculum	instable at room temperature

9. Format of the test	
9.1 Number of plants per genotype	20
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible	Daniela
Resistant tomato	Matias
9.5 Test facility	glasshouse
9.6 Temperature	23°C during the day; 21°C during the night
9.7 Light	16 hours
10. Inoculation	
10.3 Plant stage at inoculation	14 days
10.4 Inoculation method	with ice-cold 0,01 M PBS pH 7 and carborundum
10.5 First observation	7 days after inoculation
10.6 Second observation	14 days after inoculation
10.7 Final observations	18 days after inoculation
11. Observations	
11.1 Method	visual
11.2 Observation scale	necrotic spots on the top leaves
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of
	resistant and susceptible controls
<ol><li>Interpretation of data in terms of</li></ol>	UPOV characteristic states
_	1] necrotic spots present
present [9	9] No symptoms
12 Critical control paints:	

present 13. Critical control points:

ToTV is transmitted by white fly (*Bemisia tabaci*). Produce inoculum with ice-cold mortar and pestle. During inoculation the temperature should be below 25°C.

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

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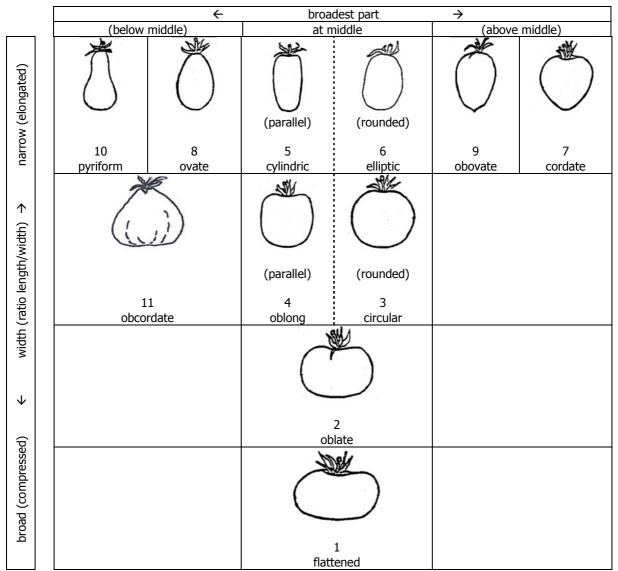
# 10. TECHNICAL QUESTIONNAIRE



	TECHNICAL QUESTIONNAIRE		
	to be completed in connection with an application for Community Plant Variety Rights Please answer all questions. A question without any answer will lead to a non-attribution of an application date. In cases where a field / question is not applicable, please state so.		
1.	Botanical taxon: Name of	the genus, species or sub-species to which the variety belongs and common name	
	[]	Solanum lycopersicum L.	
	[]	Solanum lycopersicum L. x Solanum pimpinellifolium L.	
		TOMATO	
2.	Applicant(s): Name(s) an name and address of the pr	d address(es), phone and fax number(s), Email address, and where appropriate ocedural representative	
3.	Variety denomination		
	a) Where appropriate prop	osal for a variety denomination:	
	b) Provisional designation (	breeder's reference):	

4.	Information on the breeding scheme and propagation of the variety			
4.1	Method of maintenance and reproduction			
	(a)	(i) hybrid	[ ]	
		(ii) open-pollinated variety	[ ]	
		(iii) parent line	[ ]	
	(b)	(i) seed propagated	[ ]	
		(ii) vegetatively propagated	[ ]	
	(c)	Other information on genetic origin and breed	ing method[ ]	
5.	corre	racteristics of the variety to be indicate esponding characteristic in the CPVO Protocol; pesponds).		
	Characteristics Example varieties Note			Note
5.1 (2)	P	Plant: growth type		
		determinate	Campbell 1327, Prisca	1[]
		indeterminate	Marmande VR, Saint-Pierre, San Marzano 2	2[]
5.2 (10)	L	eaf: type of blade		
		pinnate	Mikado, Pilot, Red Jacket	1[]
		bipinnate	Lukullus, Saint-Pierre	2[]
5.3 (19)	P	eduncle: abscission layer		
		absent	Aledo, Bandera, Count, Lerica	1[]
		present	Montfavet H 63.5, Roma	9[]
5.4 (21)	F	ruit: green shoulder (before maturity)		
		absent	Felicia, Rio Grande, Trust	1[]

	Characteristics	Example varieties	Note
5.5 (25)	Fruit: green stripes (before maturity)		
	absent	Daniela	1[]
	present	Green Zebra, Tigerella	9[]
5.6 (26)	Fruit: size		
	very small	Cerise, Sweet 100	1[]
	very small to small		2[]
	small	Early Mech, Europeel, Roma	3[]
	small to medium		4[]
	medium	Alphamech, Diego	5[]
	medium to large		6[]
	large	Carmello, Ringo	7[]
	large to very large		8[]
	very large	Erlidor, Lydia, Muril	9[]
5.7 (28)	Fruit: shape in longitudinal section		
	flattened	Campbell 28, Marmande VR	1[]
	oblate	Montfavet H 63.4, Montfavet H 63.5	2[]
	circular	Cerise, Moneymaker	3[]
	oblong	Early Mech, Peto Gro	4[]
	cylindric	Hypeel 244, Macero II, San Marzano 2	5[]
	elliptic	Alcaria, Castone	6[]
	cordate	Valenciano	7[]
	ovate	Barbara, Dualrow, Soto	8[]
	obovate	Duquesta, Estelle Rimone, Rio Grande	9[]
	pyriform	Europeel	10 [ ]
	obcordate	Cuore del Ponente, Magno	11 [ ]



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

	Characteristics	Example varieties	Note
5.8 (36)	Fruit: number of locules		
	only two	Early Mech, Europeel, San Marzano	1[]
	two and three	Alphamech, Futuria	2[]
	three and four	Montfavet H 63.5	3[]
	four, five or six	Raïssa, Tradiro	4[]
	more than six	Marmande VR	5[]

	Characteristics	Example varieties	Note
5.9 (37)	Fruit: colour at maturity		
	cream	Jazon, White Mirabell	1[]
	yellow	Goldene Königin, Yellow Pear	2[]
	orange	Sungold	3[]
	pink	House Momotaro	4[]
	red	Daniela, Ferline, Montfavet H 63.5	5[]
	brown	Ozyrys	6[]
	green	Green Grape, Green Zebra	7[]
5.10 (43)	Resistance to <i>Meloidogyne incognita</i> (Mi)		
	susceptible	Casaque Rouge, Clairvil	1[]
	moderately resistant	Campeon	2[]
	highly resistant	Anabel, Anahu	3[]
5.11 (44)	Resistance to <i>Verticillium</i> sp. (Va and Vd) —	Race 0	
	absent	Anabel, Marmande verte	1[]
	present	Clairbil, Marmande VR	9[]
5.12 (45.1)	Resistance to Fusarium oxysporum f. sp. lyc	copersici (Fol) Race 0 (ex1)	
	absent	Marmande verte	1[]
	present	Anabel, Marporum, Marsol	9[]
5.13 (45.2)	Resistance to Fusarium oxysporum f. sp. lyc	copersici (Fol) Race 1 (ex2)	
	absent	Marmande verte	1[]
	present	Motelle, Walter	9[]
5.14 (48.1)	Resistance to Tomato mosaic virus (ToMV) -	- Strain 0	
	absent	Monalbo	1[]
	present	Mobaci, Mocimor, Moperou	9[]

	Chara	cteristics	Example varieties	Note					
5.15 (55)									
	absent		Montfavet H 63.5	1[]					
	present		Lisboa	9[]					
6.	6. Similar varieties and differences from these varieties:  Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.								
Denomination of similar variety		Characteristic in which the similar variety is different <sup>1)</sup>	State of expression of similar variety	State of expression of candidate variety					

In the case of identical states of expressions of both varieties, please indicate the size of the difference

7.	Additional information which may help in the examination of the variety					
7.1	Resistance to pests and diseases					
Resist	rance to :	absent	present	not tested		
a) Fusarium oxysporum f. sp. lycopersici (Fol) Race 2 (ex 3)(char. 45.3)		[ ]	[ ]	[ ]		
b) <i>Fu</i> s	sarium oxysporum f. sp. radicis-lycopersici (Forl) (char. 46)	[ ]	[ ]	[ ]		
c) <i>Fui</i>	lvia fulva Ff):					
(i) R	ace 0 (char. 47.1)	[ ]	[]	[]		
(ii) Group A (char. 47.2)		[ ]	[]	[ ]		
(iii)	Group B (char. 47.3)	[ ]	[]	[ ]		
(iv)	Group C (char. 47.4)	[ ]	[]	[ ]		
(v) (	Group D (char. 47.5)	[ ]	[]	[ ]		
(vi)	Group E (char. 47.6)	[ ]	[ ]	[ ]		
d) To	mato mosaic virus (ToMV)					
(i) S	Strain 1 (char. 48.2)	[ ]	[ ]	[ ]		
(ii) S	Strain 2 (char. 48.3)	[ ]	[ ]	[ ]		
e) <i>Ph</i>	ytophtora infestans (Pi) (char. 49)	[ ]	[ ]	[ ]		
f) <i>Pyr</i>	renochaeta lycopersici (PI) (char. 50)	[ ]	[ ]	[ ]		
g) <i>Ste</i>	emphylium spp. (char. 51)	[ ]	[ ]	[]		
h) <i>Pse</i>	eudomonas syringae pv. tomato (Pst) (char. 52)	[ ]	[ ]	[ ]		
i) <i>Ral</i> s	stonia solanacearum race 1 (Rs) (char. 53)	[ ]	[ ]	[ ]		
j) Ton	nato yellow leaf curl virus (TYLVC) (char. 54)	[ ]	[ ]	[ ]		
k) <i>Le</i> ı	veillula taurica (Lt) (char. 56)	[ ]	[ ]	[ ]		
l) <i>Oid</i>	ium neolycopersici (On) (ex <i>Oidium lycopersicum</i> (OI) (char. 57)	[ ]	[ ]	[ ]		
m) To	omato torrado virus (ToTV) (char. 58)	[ ]	[ ]	[ ]		
n) Otł	ners (please specify)	[ ]	[ ]	[]		

7.2	In addition to the information provided in sections 5 and 6, are there any other characteristics which may help to distinguish the variety?					
	[ ] YES, please specify					
	[ ] NO					
7.3	Are there any special conditions for growin	g the variety or conducting the examination?				
7.3.1	Type of culture					
	- greenhouse	[ ]				
	- in the open	[ ]				
7.3.2 N	Main use					
	- fresh market or garden	[ ]				
	- industrial processing (indicate type)	[ ]				
	- pot plant	[ ]				
	- rootstock	[ ]				
7.3.3.	Other conditions					
	[ ] YES, please specify					
	[ ] NO					
7.4	Other information					
	[ ] YES, please specify					
	[ ] NO					

7.5	Photo					
	is recommended to provide a representative colour image of the variety to accompany the Technical uestionnaire					
8.	GMO-information required					
	The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive EC/2001/18 of 12/03/2001.					
	[ ] YES [ ] NO					
	If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation does not pose risks to the environment according to the norms of the above-mentioned Directive.					
9.	Information on plant material to be examined					
	<b>9.1</b> The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.					
	<b>9.2</b> 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 the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:					
	(a) Microorganisms (e.g. virus, bacteria, phytoplasma)	[	] Yes	[	] No	
	(b) Chemical treatment (e.g. growth retardant or pesticide)	[	] Yes	[	] No	
	(c) Tissue culture	[	] Yes	[	] No	
	(d) Other factors	[	] Yes	[	] No	
	Please provide details of where you have indicated "Yes":					

### 10. Possible place of the technical examination

In case the CPVO needs to arrange a technical examination for this candidate variety, there might be more than one examination office entrusted by the CPVO suitable to grow your variety. In this case, the Office will decide on the place of the technical examination but you might wish to express here a preference in respect of an examination office. The available entrusted examination offices for that species can be found in the S2 Gazette under

http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date Signature Name

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