



## **PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY**

***Capsicum annuum* L.**

**SWEET PEPPER, HOT PEPPER, PAPRIKA, CHILI**

UPOV Code: CAPSI\_ANN

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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 *Capsicum annuum* 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/export/sites/upov/resource/en/tg\\_1\\_3.pdf](http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf)), its associated TGP documents (<http://www.upov.int/tgp/en/>) and the relevant UPOV Test Guideline TG/76/8 Rev.2 dated 20/09/2018 (<https://www.upov.int/edocs/tgdocs/en/tg076.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability.

### **1.2 Entry into Force**

The present protocol enters into force on **01.04.2020**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

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

### **1.3 Reporting between Examination Office and CPVO and Liaison with Applicant**

#### **1.3.1 Reporting between Examination Office and CPVO**

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

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

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

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

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

#### **1.3.2 Informing on problems in the DUS test**

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

#### **1.3.3 Sample keeping in case of problems**

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

## **2. MATERIAL REQUIRED**

### **2.1 Plant material requirements**

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <http://cpvo.europa.eu/applications-and-examinations/technical-examinations/submission-of-plant-material-s2-publication> in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

## **2.2 Informing the applicant of plant material requirements**

The CPVO informs the applicant that

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

## **2.3 Informing about problems on the submission of material**

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

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

## **3. METHOD OF EXAMINATION**

### **3.1 Number of growing cycles**

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

### **3.2 Testing Place**

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness" [http://www.upov.int/edocs/tgpdocs/en/tgp\\_9.pdf](http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf).

### **3.3 Conditions for Conducting the Examination**

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

### **3.4 Test design**

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

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

### **3.5 Special tests for additional characteristics**

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

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

### **3.6 Constitution and maintenance of a variety collection**

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

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

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

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

### 3.6.1 Forms of variety collection

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

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

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

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

### 3.6.2 Living Plant Material

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

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

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

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

### 3.6.3 Range of the variety collection

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

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

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

### 3.6.5 Maintenance and renewal/update of a living variety collection

#### **(a) Seed propagated species**

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

#### **(b) Vegetatively propagated species**

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

## **4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY**

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

### **4.1 Distinctness**

#### 4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' ([http://www.upov.int/edocs/tgpdocs/en/tgp\\_9.pdf](http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf)) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

#### 4.1.2 Consistent differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

#### 4.1.3 Clear differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

#### 4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 20 plants or parts taken from each of 20 plants and any other observations made on all plants in the test, disregarding any off-type plants.

In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 20.

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

#### 4.1.5 Method of observation

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

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

Type of observation: visual (V) or measurement (M)

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

Type of record: for a group of plants (G) or for single, individual plants (S)

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

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

## 4.2 **Uniformity**

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' ([http://www.upov.int/edocs/tgpdocs/en/tgp\\_10.pdf](http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf)) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical.

### **(a) Cross-pollinated varieties**

The assessment of uniformity should be according to the recommendations for cross-pollinated varieties in the UPOV-General Introduction to DUS.

For the assessment of uniformity of open-pollinated varieties, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 20 plants, 2 off-types are allowed.

### **(b) Hybrid varieties**

For the assessment of uniformity of hybrids, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 20 plants, 1 off-type is allowed.

### 4.3 Stability

- 4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' ([http://www.upov.int/edocs/tgpdocs/en/tgp\\_11.pdf](http://www.upov.int/edocs/tgpdocs/en/tgp_11.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 **Technical Protocols covering both seed-propagated and vegetatively propagated varieties**

Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

## 5. GROUPING OF VARIETIES AND ORGANISATION OF THE GROWING TRIAL

- 5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

- 5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organise the growing trial so that similar varieties are grouped together.

- 5.3 The following have been agreed as useful grouping characteristics.

- a) Seedling: anthocyanin coloration of hypocotyl (characteristic 1)
- b) Plant: shortened internode (in upper part) (characteristic 3)
- c) Fruit: colour (before maturity) (characteristic 20)
- d) Fruit: shape in longitudinal section (characteristic 27)
- e) Fruit: colour (at maturity) (characteristic 32)
- f) Fruit: capsaicin in placenta (characteristic 44)
- g) Resistance to *Tobamovirus*, *Tobacco Mosaic Virus* Pathotype 0 (TMV: P0) (characteristic 47.1)
- h) Resistance to *Tobamovirus*, *Pepper Mild Mottle Virus* Pathotype 1-2 (PMMoV: 1-2) (characteristic 47.2)
- i) Resistance to *Tobamovirus*, *Pepper Mild Mottle Virus* Pathotype 1-2-3 (PMMoV: 1-2-3) (characteristic 47.3)
- j) Resistance to *Potato Y Virus* Pathotype 0 (PVY: 0) (characteristic 48.1)
- k) Resistance to *Tomato spotted wilt virus* Pathotype 0 (TSWV: 0) (characteristic 51)

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

- 5.5 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

## 6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

### 6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted or by specific legislation on plant health. In the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

#### Asterisked characteristics

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

### 6.2. States of expression and corresponding notes

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

State	Note
small	3
medium	5
large	7

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

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

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

### 6.3 Example Varieties

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



## 6.4 Legend

### For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2
(*)	Asterisked characteristic	-see Chapter 6.1.2

### For column 'UPOV N°':

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

(*)	UPOV Asterisked characteristic	-Characteristics that are important for the international harmonization of variety descriptions.
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### For column 'Stage, method':

MG, MS, VG, VS		-see Chapter 4.1.5
(a)-{x}	Explanations covering several Characteristics	-see Chapter 8.1

## 7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>1.</b>	<b>1. (*)</b>	<b>VG</b>	<b>Seedling: anthocyanin coloration of hypocotyl</b>		
<b>QL</b>			absent	Albaregia, Albena	1
<b>G</b>			present	Lamuyo	9
<b>2. (+)</b>	<b>2.</b>	<b>VG/MS</b>	<b>Plant: length of stem</b>		
<b>QN</b>			short	Delphin, Trophy	3
			medium	Belsir, Lamuyo	5
			long	Lipari, Marconi, Rouge long ordinaire	7
<b>3. (+)</b>	<b>4. (*)</b>	<b>VG</b>	<b>Plant: shortened internode (in upper part)</b>		
<b>QL</b>			absent	California wonder, De Cayenne	1
<b>G</b>			present	Fehér, Kalocsai 601, Kalocsai 702	9
<b>4. (+)</b>	<b>5.</b>	<b>MS</b>	<b><u>Varieties with shortened internodes only:</u> Plant: number of internodes between the first flower and shortened internodes</b>		
<b>QN</b>			none	Kalocsai 601	1
			one to three	Fehér	2
			more than three	Kalocsai 702	3
<b>5.</b>	<b>6.</b>	<b>VG/MS</b>	<b><u>Varieties without shortened internodes only:</u> Plant: length of internodes (on primary side shoots)</b>		
<b>QN</b>			very short	Albaregia	1
			short	Bandero, Blondy, Danubia, Tenor	3
			medium	Dolmi, Florian, Órias	5
			long	Corno di toro rosso	7
			very long	Fenice, Kalocsai M, Sienor	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
6.	7.	VG	<b>Plant: anthocyanin coloration of nodes</b>			
				absent	Albaregia	1
QL			present	California wonder	9	
7.	8.	VG	<b>Stem: intensity of anthocyanin coloration of nodes</b>			
				very weak		1
				weak	California wonder, Clio, Doux d'Espagne, Doux très long des Landes, Golden calwonder	3
				medium	Clovis, Lamuyo, Sonar	5
				strong	Piquant d'Algérie, Zarai	7
			very strong	Alwin, Koral, Lito, Pusztagold	9	
8.	9.	VG	<b>Stem: hairiness of nodes</b>			
				absent or very weak	Arlequin	1
				weak	Andevalo, Clovis	3
				medium	Doux très long des Landes, Farnese	5
				strong	Fenice, Solario	7
			very strong	Mino	9	
9. (+)	10.	VG/MS	<b>Plant: height</b>			
				very short	Kalocsai 601	1
				short	Albaregia	3
				medium	HRF	5
				tall	Century, Orias	7
QN			very tall	Hot chili	9	

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>10.</b>	<b>11.</b>	<b>VG/MS</b>	<b>Leaf: length of blade</b>		
<b>QN</b>			very short	Macska sárga, Tüzes piros	1
			short	De Cayenne, Szentesi cseresznye	3
			medium	Atol, Blondy, Marconi, Merit, Anthea	5
			long	Cupido, Dolmy, Encore, Mazurka, Monte	7
			very long	Predi, Solario	9
<b>11.</b>	<b>12.</b>	<b>VG/MS</b>	<b>Leaf: width of blade</b>		
<b>QN</b>			very narrow	Macska sárga, Recio, Tüzes piros	1
			narrow	De Cayenne, Pusztagold, Szentesi cseresznye	3
			medium	Albaregia, Balaton, Danubia, Marconi, Merit	5
			broad	California wonder, Golden calwonder, Sienor, Solario	7
<b>12.</b>	<b>13.</b>	<b>VG</b>	<b>Leaf: intensity of green colour</b>		
<b>QN</b>			very light	Amaryllis, Lombardo	1
			light	Piquant d'Algérie, Pusztagold	3
			medium	Doux très long des Landes, Merit	5
			dark	Dolmy, Tinto	7
			very dark	Hot Chilli, Recio, Soleor	9
<b>13. (+)</b>	<b>14.</b>	<b>VG</b>	<b>Leaf: shape</b>		
<b>PQ</b>			lanceolate	Diavolo, Recio	1
			ovate	Balico, Sonar	2
			broad elliptic	Solario	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>14.</b> <b>QN</b>	<b>15.</b>	<b>VG</b>	<b>Leaf: undulation of margin</b>		
			absent of very weak	De Cayenne	1
			weak	Doux très long des Landes	3
			medium	Tenor	5
			strong	Sucette de Provence, Tosca	7
	very strong	Farya	9		
<b>15.</b> <b>QN</b>	<b>16.</b>	<b>VG</b>	<b>Leaf: blistering</b>		
			very weak	Century, Recio, Sofiane	1
			weak	Pusztagold	3
			medium	Merit	5
			strong	Greygo, PAZ pallagi	7
	very strong	Florian	9		
<b>16.</b> <b>(+)</b> <b>QN</b>	<b>17.</b>	<b>VG</b>	<b>Leaf: profile in cross section</b>		
			strongly concave	Slávy	1
			moderately concave	Doux italien, Favolor	3
			flat	De Cayenne, Recio	5
			moderately convex	Albaregia	7
	strongly convex	Tinto	9		
<b>17.</b> <b>QN</b>	<b>18.</b>	<b>VG</b>	<b>Leaf: glossiness</b>		
			very weak	Diavolo	1
			weak	De Cayenne, Doux très long des Landes	3
			medium	Alby, Eolo	5
			strong	Andevalo, Floridor	7
	very strong	Cubor, Petit Marseillais	9		

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>18. (+)</b>	<b>19. (* )</b>	<b>VG</b>	<b>Peduncle: attitude</b>		
<b>QN</b>			erect	Fehér, Red Chili	1
			semi-drooping	Blondy	2
			drooping	Heldor, Lamuyo	3
<b>19.</b>	<b>20.</b>	<b>VG</b>	<b>Flower: anthocyanin coloration in anther</b>		
<b>QL</b>			absent	Danza	1
			present	Lamuyo	9
<b>20.</b>	<b>21. (* )</b>	<b>VG</b>	<b>Fruit: colour (<u>before</u> maturity)</b>		
<b>PQ</b>		<b>(a)</b>	greenish white	Blanc d'Espagne	1
			yellow	Fehér, Sweet banana	2
			green	California wonder, Lamuyo	3
<b>G</b>			purple	Nigra	4
<b>21.</b>	<b>22.</b>	<b>VG</b>	<b>Fruit: intensity of colour (<u>before</u> maturity)</b>		
<b>QN</b>		<b>(a)</b>	very light		1
			light		3
			medium		5
			dark		7
			very dark		9
<b>22.</b>	<b>23.</b>	<b>VG</b>	<b>Fruit: anthocyanin coloration (<u>before</u> maturity)</b>		
<b>QL</b>		<b>(a)</b>	absent	Lamuyo	1
			present	Alabástrom, Purple beauty	9
<b>23.</b>	<b>24.</b>	<b>VG</b>	<b>Fruit: attitude</b>		
<b>QN</b>		<b>(b)</b>	erect	Kalocsai 601, Red Chili	1
			horizontal	PAZ szentesi, Vinedale	2
			drooping	De Cayenne, Lamuyo	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>24.</b> <b>QN</b>	<b>25.</b>	<b>VG/MS</b> <b>(b)</b>	<b>Fruit: length</b>		
			very short	Cherry Sweet, Topgirl	1
			short	Delphin, Petit carré doux	3
			medium	Fehér, Lamuyo	5
			long	Doux d'Espagne, Majister	7
			very long	Arabal, Corno di toro, Marconi	9
<b>25.</b> <b>QN</b>	<b>26.</b>	<b>VG/MS</b> <b>(b)</b>	<b>Fruit: diameter</b>		
			very narrow	De Cayenne, Recio	1
			narrow	Doux très long des Landes	3
			medium	Doux italien, Corno di toro	5
			broad	Clovis, Lamuyo	7
			very broad	Floridor, Ibleor, Inca, Joly rosso, Quadrato d'Asti, Surpas	9
<b>26.</b> <b>QN</b>	<b>27.</b> <b>(*)</b>	<b>MS</b> <b>(b)</b>	<b>Fruit: ratio length/diameter</b>		
			very small	Liebesapfel, PAZ szentesi, Rotopa	1
			small	Bucano, Topgirl	3
			medium	Adra, Cherry Sweet, Daniel, Delphin, Edino	5
			large	Heldor, Lamuyo, Magister, Tenno, Vidi	7
			very large	De Cayenne, Kusamon, Spadi	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>27. (+)</b>	<b>28. (*)</b>	<b>VG</b>	<b>Fruit: shape in longitudinal section</b>		
<b>PQ</b>		<b>(b)</b>	oblate	Liebesapfel, PAZ szentesi, Topepo rosso	1
			circular	Cherry Sweet	2
			cordate	Daniel	3
			square	Delphin, Yolo Wonder	4
			rectangular	Clovis, Nocera rosso	5
			trapezoidal	Delta, Piperade	6
			moderately triangular	Fehér, Marconi	7
			narrowly triangular	De Cayenne, Demon	8
<b>G</b>			horn shaped	Tauro	9
<b>28.</b>	<b>29.</b>	<b>VG</b>	<b>Fruit: shape in cross section (at level of placenta)</b>		
<b>PQ</b>		<b>(b)</b>	elliptic	Sweet banana	1
			angular	Vinedale	2
			circular	Cherry Sweet, Doux très long des Landes	3
<b>29. (+)</b>	<b>30.</b>	<b>VG</b>	<b>Fruit: sinuation of pericarp at basal part</b>		
<b>QN</b>		<b>(b)</b>	absent or very weak	Delphin, Kalocsai V-2, Milord	1
			weak	Donat	3
			medium	Duna, Banán	5
			strong	Alfa	7
			very strong	Édes spiral, Doux italien	9
<b>30. (+)</b>	<b>31.</b>	<b>VG</b>	<b>Fruit: sinuation of pericarp excluding basal part</b>		
<b>QN</b>		<b>(b)</b>	absent or very weak	Delphin, Milord	1
			weak	Clovis, Sonar	3
			medium	Ursus	5
			strong	De Cayenne, Doux italien	7
			very strong	Arabal	9



CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>31.</b>	<b>32.</b> (*)	<b>VG</b>	<b>Fruit: texture of surface</b>		
<b>QN</b>		<b>(b)</b>	smooth or very wrinkled	Milord	1
			slightly wrinkled	Doux très long des Landes	2
			strongly wrinkled	Sierra Nevada	3
<b>32.</b>	<b>33.</b> (*)	<b>VG</b>	<b>Fruit: colour (<u>at</u> maturity)</b>		
<b>PQ</b>		<b>(b)</b>	yellow	Golden calwonder, Heldor	1
			orange	Ariane	2
			red	Fehér, Lamuyo	3
			brown	Brupa, Negral	4
<b>G</b>			green	Green6203	5
<b>33.</b>	<b>34.</b>	<b>VG</b>	<b>Fruit: intensity of colour (<u>at</u> maturity)</b>		
<b>QN</b>		<b>(b)</b>	light		3
			medium		5
			dark		7
<b>34.</b>	<b>35.</b>	<b>VG</b>	<b>Fruit: glossiness</b>		
<b>QN</b>		<b>(b)</b>	very weak	Macska sárga, Pikanta	1
			weak	Doux très long des Landes	3
			medium	Carré doux extra hâtif, Lamuyo, Sonar	5
			strong	Doux italien, Trophy	7
			very strong	Floridor, Kappy	9
<b>35.</b>	<b>36.</b> (*)	<b>VG</b>	<b>Fruit: stalk cavity</b>		
<b>QL</b>		<b>(b)</b>	absent	Corinto, Corno di toro, Sweet banana, Sucette de Provence	1
			present	Bingor, Lamuyo	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>36.</b> <b>QN</b>	<b>37.</b>	<b>VG</b> <b>(b)</b>	<b>Fruit: depth of stalk cavity</b>		
			very shallow	Flush, Kaméleon, Niagara	1
			shallow	Delphin, Doux italien, Fehér, Latino	3
			medium	Lamuyo, Magister	5
			deep	Osir, Quadrato d'Asti rosso, Surpas	7
			very deep	Cancun, Cubor, Pablor, Shy Beauty	9
<b>37.</b> <b>PQ</b>	<b>38.</b>	<b>VG</b> <b>(b)</b>	<b>Fruit: shape of apex</b>		
			very acute	De Cayenne, Hot Chilli	1
			moderately acute		2
			rounded	Cherry Sweet	3
			moderately depressed	Quadrato d'Asti rosso	4
			very depressed	Kerala, Monte, Osir	5
<b>38.</b> <b>(+)</b> <b>QN</b>	<b>39.</b>	<b>VG</b> <b>(b)</b>	<b>Fruit: depth of interloculary grooves</b>		
			absent very shallow	De Cayenne	1
			shallow	Milord, Topgirl	3
			medium	Clovis, Lamuyo, Marconi	5
			deep	Majister, Surpas	7
<b>39.</b> <b>QN</b> <b>G</b>	<b>40.</b> <b>(*)</b>	<b>MG</b> <b>(b)</b>	<b>Fruit: number of locules</b>		
			predominantly two	De Cayenne	1
			equally two and three	Fehér	2
			predominantly three	Century	3
			equally three and four	Lamuyo, Sonar	4
			predominantly four and more	Palio, PAZ szentesi	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>40.</b>	<b>41.</b> (*)	<b>VG</b>	<b>Fruit: thickness of flesh</b>		
<b>QN</b>		<b>(b)</b>	very thin	De Cayenne, Macska sárga, Petit marseillais, Recio	1
			thin	Banán, Carré doux extra hâtif, Doux très long des Landes	3
			medium	Fehér, Lamuyo	5
			thick	Andevalo, Bingor, Daniel, Topgirl	7
			very thick	Dragox Roda, Regolo, Solario	9
<b>41.</b>	<b>42.</b>	<b>VG/MS</b>	<b>Stalk: length</b>		
<b>QN</b>		<b>(b)</b>	very short	Greygo, Golden calwonder	1
			short	Surpas, Yolo Wonder, Zenith	3
			medium	Fehér, Sonar	5
			long	De Cayenne, Sierra Nevada, Sweet banana	7
			very long	Farnese, Lipari, Oasis	9
<b>42.</b>	<b>43.</b>	<b>VG/MS</b>	<b>Stalk: thickness</b>		
<b>QN</b>		<b>(b)</b>	very thin	De Cayenne, Doux très long des Landes, Macska sárga, Recio	1
			thin	Sweet banana	3
			medium	Doux italien, Surpas	5
			thick	Lamuyo, Trophy Palio	7
			very thick	Domingo, Galaxy, Paraiso	9
<b>43.</b> (+)	<b>44.</b>	<b>VG</b>	<b>Calyx: aspect</b>		
		<b>(b)</b>	non enveloping	Lamuyo, Sonar	1
<b>QL</b>			enveloping	De Cayenne, Sweet banana	2
<b>44.</b> (+)	<b>45.</b> (*)	<b>VG</b>	<b>Fruit: capsaicin in placenta</b>		
<b>QL</b>		<b>(b)</b>	absent	Sonar	1
<b>G</b>			present	De Cayenne	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>45.</b>	<b>46.</b>	<b>VG</b>	<b>Time of beginning of flowering (first flower on second flowering node)</b>		
<b>QN</b>			early	Carré doux extra hâtif, Cupido, Fehér, Flaviano, Lito, Trophy	3
			medium	Lamuyo, Latino	5
			late	Daniel, Piquant d'Algérie, Zingaro	7
<b>46. (+)</b>	<b>47.</b>	<b>VG</b>	<b>Time of maturity</b>		
<b>QN</b>			very early	Koral, Macska sárga, Madison	1
			early	Fehér, Lady Bell, Topgirl	3
			medium	Lamuyo, Latino, Sonar	5
			late	Daniel, Doux d'Espagne	7
			very late	Cancun, California wonder	9
<b>47. (+)</b>	<b>48.</b>		<b>Resistance to <i>Tobamovirus</i></b>		
<b>47.1 (*)</b>	<b>48.1 (*)</b>	<b>VG</b>	<b><i>Tobacco mosaic virus</i> Pathotype 0 (TMV: 0)</b>		
<b>QL</b>			absent	Lamu, Pepita, Piquillo	1
<b>G</b>			present	Fehérozön, Turia, Yolo Wonder	9
<b>47.2 (*)</b>	<b>48.2 (*)</b>	<b>VG</b>	<b><i>Pepper mild mottle virus</i> Pathotype 1-2 (PMMoV: 1-2)</b>		
<b>QL</b>			absent	Fehérozön, Lamu, Turia, Yolo Wonder	1
<b>G</b>			present	Candela, Ferrari, Novi 3, PI15225	9
<b>47.3 (*)</b>	<b>48.3 (*)</b>	<b>VG</b>	<b><i>Pepper mild mottle virus</i> Pathotype 1-2-3 (PMMoV: 1-2-3)</b>		
<b>QL</b>			absent	Candela, Ferrari, Yolo Wonder	1
<b>G</b>			present	Bisonte, Friendly, Tom 4	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>48. (+)</b>	<b>49.</b>		<b>Resistance to <i>Potato Y Virus</i> (PVY)</b>		
<b>48.1 (*)</b>	<b>49.1 (*)</b>	<b>VG</b>	<b>Pathotype 0 (PVY: 0)</b>		
<b>QL</b>			absent	Ferrari, Piquillo, Yolo Wonder	1
<b>G</b>			present	Andalus, Vidi, Yolo Y	9
<b>48.2</b>	<b>49.2</b>	<b>VG</b>	<b>Pathotype P1 (PVY: 1)</b>		
<b>QL</b>			absent	Yolo Wonder, Yolo Y	1
			present	Florida VR2	9
<b>48.3</b>	<b>49.3</b>	<b>VG</b>	<b>Pathotype P1-2 (PVY: 1-2)</b>		
<b>QL</b>			absent	Florida VR2, Yolo Wonder, Yolo Y	1
			present	Serrano Criollo de Morelos 334	9
<b>49. (+)</b>	<b>50.</b>	<b>VG</b>	<b>Resistance to <i>Phytophthora capsici</i> (Pc)</b>		
<b>QL</b>			absent	Yolo Wonder	1
			present	Chistera, Favolor, Phyo 636, Solario	9
<b>50. (+)</b>	<b>51.</b>	<b>VG</b>	<b>Resistance to <i>Cucumber mosaic virus</i> (CMV)</b>		
<b>QL</b>			absent	Yolo Wonder	1
			present	Alby, Favolor	9
<b>51. (+)</b>	<b>52.</b>	<b>VG</b>	<b>Resistance to <i>Tomato spotted wilt virus</i> Pathotype 0 (TSWV: 0)</b>		
<b>QL</b>			absent	Yolo Wonder	1
<b>G</b>			present	Galileo, Jackal, Jackpot	9
<b>52. (+)</b>	<b>53.</b>	<b>VG</b>	<b>Resistance to <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (Xcv)</b>		
<b>QL</b>			absent	Fehérözön, Yolo Wonder	1
			present	Aladin, Camelot, ECR-20R, Kaldóm, Kalorez, Lancelot, Pasa	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:

- Fruit characteristics which should be examined before maturity, i.e. before the first colour change.
- Fruit characteristics which should be examined at maturity, i.e. after the time of the first colour change.

### 8.2 Explanations for individual characteristics

Ad. 2: Plant: length of stem

The length of the stem is measured from the cotyledons to the first flower branch.

Ad. 3: Plant: shortened internode (in upper part)

Ad. 4: Varieties with shortened internodes only: Plant: number of internodes between flower and shortened internodes

The tests should be done on plants which have not been pruned. The shoot system of pepper consists of main stems, which are branched off from the main axis and side shoots. Two growth types of the main stems can be distinguished:

Growth type A: the main stems grow indeterminately; one or two flowers develop per node and shortened internodes never develop.

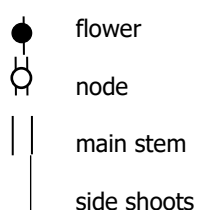
Growth type B: after the first branching of the main axis, shorter internodes appear and the growth of the main stem ends in a bunch of flowers (it appears as if there are more than two flowers per node).

Side shoots develop from the nodes on the main axis and on the main stems.

Growth type A

Growth type B

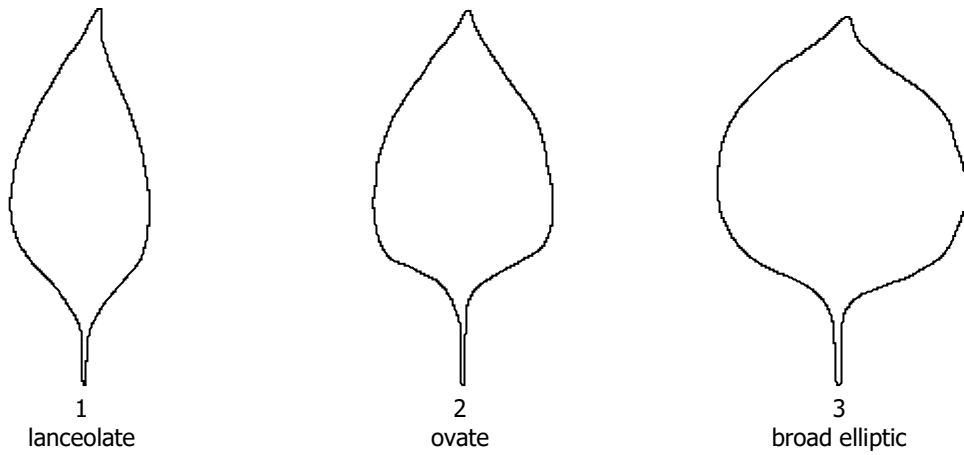
Char. 3: Plant: shortened internodes (in upper part)			
absent	present		
Char. 4: <u>Varieties with shortened internodes only:</u>	none	one to three	more than three
Plant: number of internodes between the first flower and shortened internodes	(1)	(2)	(3)



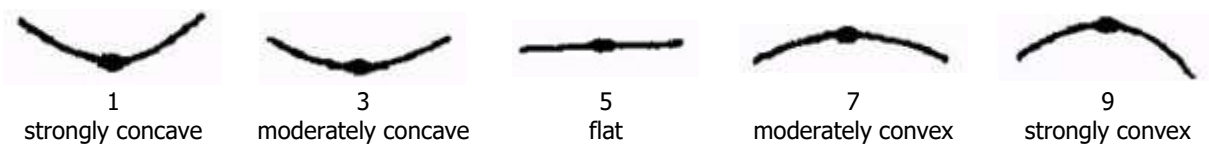
Ad. 9: Plant: height

To be observed after a fruit set on several nodes. Poor fruit set may influence the vigour and thus the height of the plant.

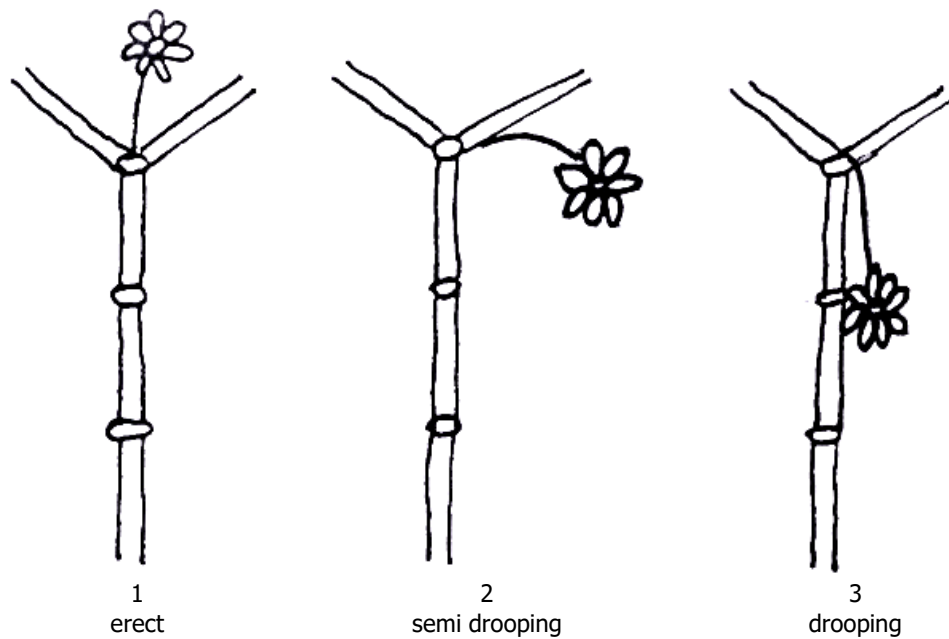
Ad. 13: Leaf: shape



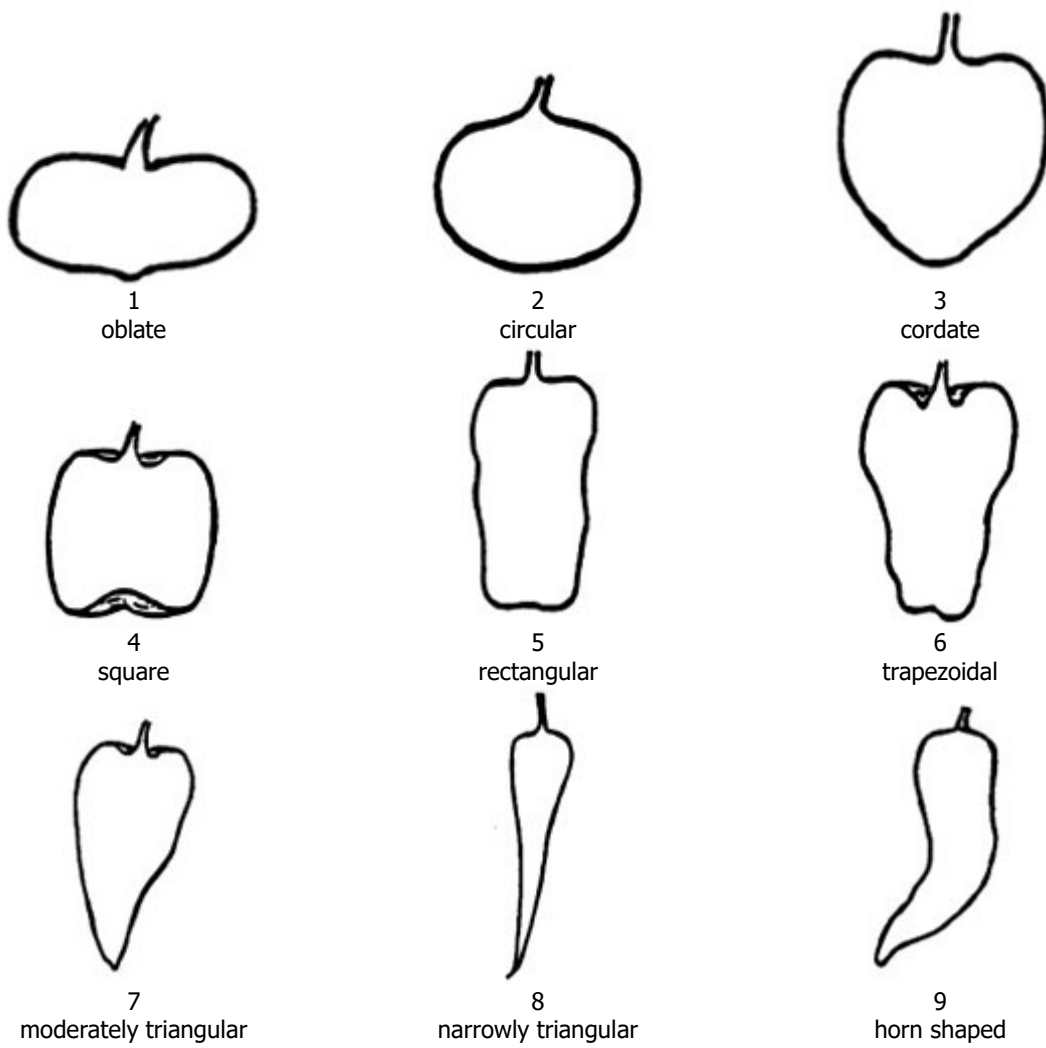
Ad. 16: Leaf: profile in cross section



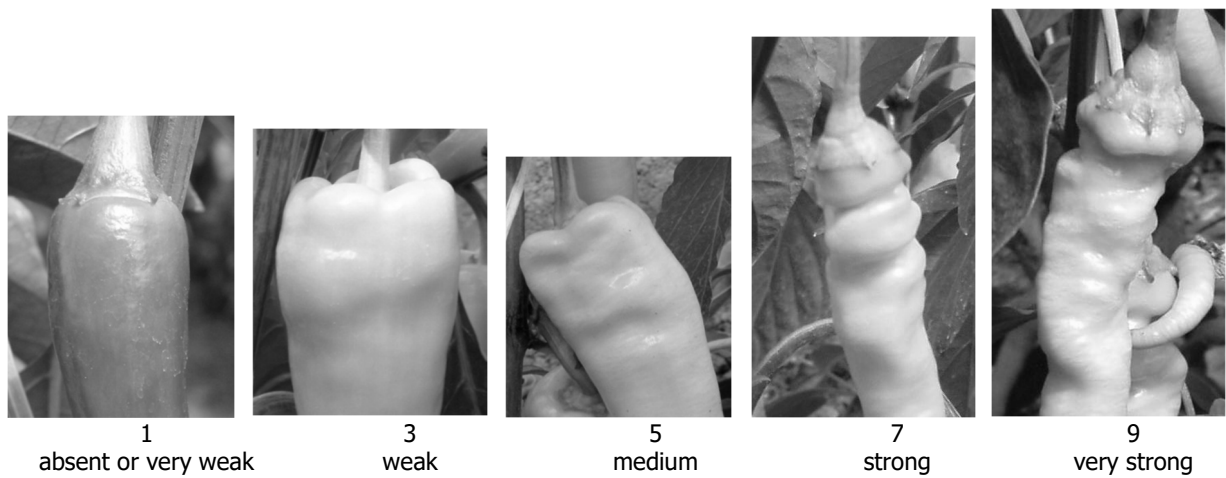
Ad. 18: Peduncle: attitude



Ad. 27: Fruit: shape in longitudinal section

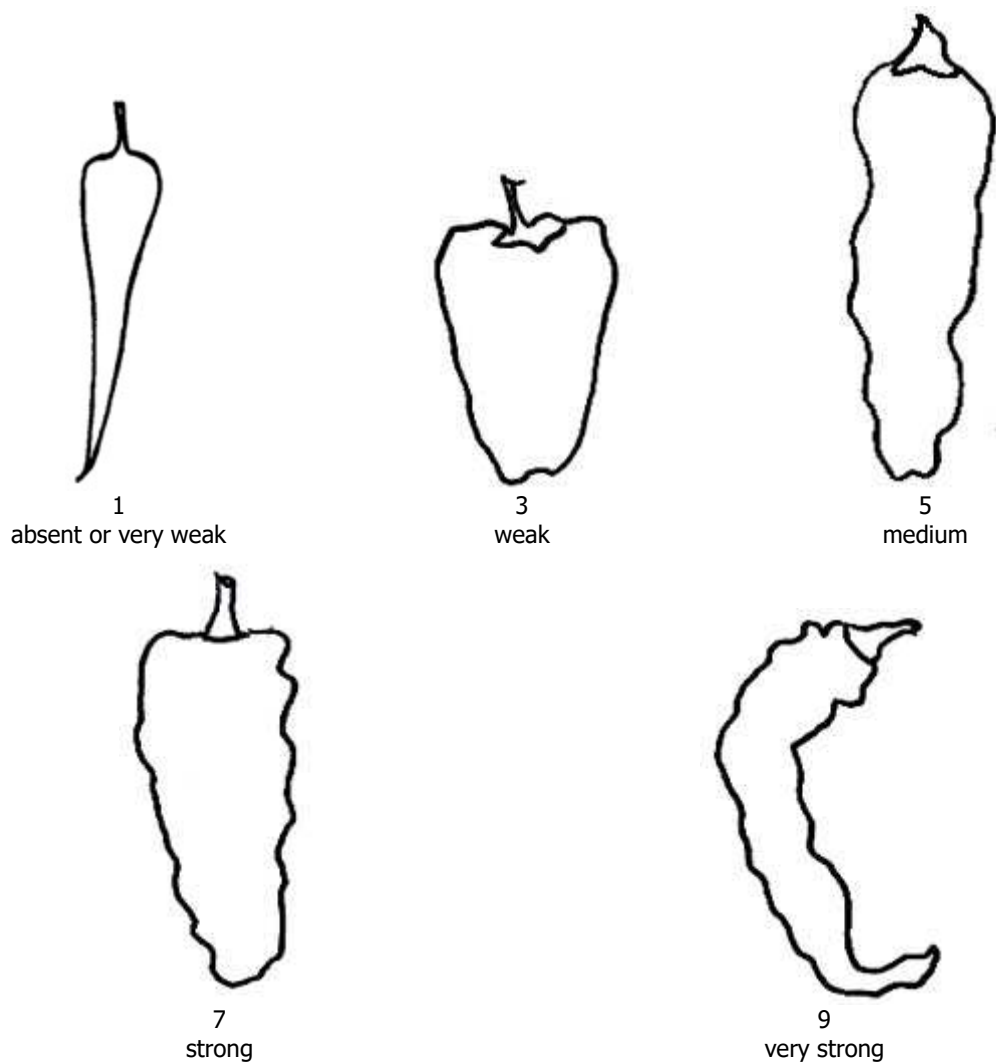


Ad. 29: Fruit: situation of pericarp at basal part





Ad. 30: Fruit: situation of pericarp excluding basal part



Ad. 38: Fruit: depth of interlocary grooves

To be observed in the middle part of the fruit.

Ad. 43: Calyx: apex



**Ad. 44: Fruit: capsaicin in placenta**

The presence of capsaicin is observed by tasting the pepper flesh together with the locules, in the placenta area.

**Ad. 46: Time of maturity**

Maturity is reached at the first colour change of the fruit.

**Ad. 47: Resistance to *Tobamovirus***

1. Pathogen..... *Tobacco mosaic virus* and *Pepper mild mottle virus*
2. Quarantine status .....no
3. Host species .....Sweet pepper, hot pepper, paprika and chili – *Capsicum annum* L.
4. Source of inoculum .....GEVES<sup>1</sup> (FR), Naktuinbouw<sup>2</sup> (NL) or INIA<sup>3</sup> (SP)
5. Isolate ..... *Tobacco mosaic virus* Pathotype 0 (TMV: 0) strain Vi-6  
*Pepper mild mottle virus* Pathotype 1-2 (PMMoV: 1-2) strain nt203  
*Pepper mild mottle virus* Pathotype 1-2-3 (PMMoV: 1-2-3) strain Eve  
The test protocols have been validated in a CPVO co-funded project<sup>4</sup> with these 3 isolates/pathotypes.
6. Establishment isolate identity .....genetically defined pepper differentials (reference ISF site: [http://www.worldseed.org/isf/differential\\_hosts.html](http://www.worldseed.org/isf/differential_hosts.html) )

	Pathotype	P0	P1	P1-2	P1-2-3
	Code	TMV: 0 ToMV: 0 TMGMV: 0 BPMoV: 0	TMV: 1 TMGMV: 1 PaMMV: 1	PMMoV: 1-2	PMMoV: 1-2-3
Variety	Gene				
Lamu, Early Calwonder	-	S	S	S	S
Tisana, Yolo Wonder	L1	R	S	S	S
Tabasco	L2	R	R	S	S
Solario F1, Novi 3, PI159236	L3	R	R	R	S
Tom4, PI260429	L4	R	R	R	R

S= susceptible; R= resistant; TMV= *Tobacco mosaic virus*; ToMV= *Tomato mosaic virus*; PMMoV= *Pepper mild mottle virus*; TMGMV= *Tobacco mild green mosaic virus*; BPMoV= *Bell pepper mottle virus*; PaMMV= *Paprika mild mottle virus*

7. Establishment pathogenicity .....Test on susceptible plants

8. Multiplication inoculum

- 8.1 Multiplication medium .....Regeneration of the virus of plant material before inoculum preparation.
- 8.2 Multiplication variety .....On susceptible pepper variety, *Tobamovirus* pathotypes may be multiplied on varieties which are selective for each particular pathotype. For TMV, because tomato and tobacco *Nicotiana tabacum* cv. Samsun have large leaves and can produce a lot of inocula, they are recommended for the multiplication of TMV: 0.
- 8.3 Plant stage at inoculation .....see 10.3
- 8.4 Inoculation medium .....see 10.1
- 8.5 Inoculation method .....see 10.4
- 8.6 Harvest of inoculum .....Symptomatic fresh leaves
- 8.7 Check of harvested inoculum....option: on young leaves of *Nicotiana tabacum* "Xanthi", check for local lesions after 5-7 days at 20-25°C.
- 8.8 Shelf life/viability inoculum.....fresh > 1 day in fridge, desiccated > 1 year in fridge or juice > 1 year in freezer at -20°C

9. Format of the test

- 9.1 Number of plants per genotype At least 20 plants.
- 9.2 Number of replicates .....-

<sup>1</sup> [matref@geves.fr](mailto:matref@geves.fr)

<sup>2</sup> [resistentie@naktuinbouw.nl](mailto:resistentie@naktuinbouw.nl)

<sup>3</sup> [resistencias@inia.es](mailto:resistencias@inia.es)

<sup>4</sup> Harmores 2 CPVO project (<http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports> )

- 9.3 Control varieties.....TMV: 0:  
Susceptible controls: Lamu, Pepita, Piquillo  
Resistant controls: Fehérözön, Yolo Wonder  
PMMoV: 1-2:  
Susceptible controls: Fehérözön, Lamu, Yolo Wonder  
Resistant controls: Ferrari, Novi 3  
PMMoV: 1-2-3:  
Susceptible controls: Ferrari, Yolo Wonder  
Resistant controls: Friendly, Tom 4  
For PMMoV: 1-2-3, it is advised to choose Ferrari as susceptible control because it is resistant to PMMoV: 1-2 or to add the differentials in tests to confirm the pathotype.
- 9.4 Test design.....add non inoculated plants
- 9.5 Test facility.....Climate room or greenhouse
- 9.6 Temperature.....20-25°C
- 9.7 Light.....12 hours or longer
- 9.8 Season.....-
- 9.9 Special measures.....-
10. Inoculation
- 10.1 Preparation inoculum.....1 g leaf with symptoms with 10 mL PBS or similar buffer or dilution of juice in water.  
Homogenize, add carborundum to buffer
- 10.2 Quantification inoculum.....-
- 10.3 Plant stage at inoculation.....TMV: 0, cotyledons to first leaf stage  
PMMoV: 1-2, cotyledon stage  
PMMoV: 1-2-3, cotyledon stage
- 10.4 Inoculation method.....rubbing with the virus suspension.
- 10.5 First observation.....TMV:0:  
4-7 days post-inoculation for observation of local necrosis.  
PMMoV: 1-2 and PMMoV: 1-2-3:  
4-7 days post-inoculation for observation of local necrotic lesions which can lead to cotyledon drop. After this date these necrosis can hardly be seen on fallen cotyledons.
- 10.6 Second observation.....TMV: 0:  
two weeks post-inoculation for observation of symptoms of susceptibility.  
PMMoV: 1-2 and PMMoV: 1-2-3:  
two weeks post-inoculation for observation of symptoms of susceptibility.
- 10.7 Final observations.....TMV:0 :  
three weeks post-inoculation.  
PMMoV: 1-2 and PMMoV: 1-2-3:  
three weeks post-inoculation.  
For TMV:0, PMMoV: 1-2 and PMMoV: 1-2-3, two of these three observations may be sufficient; the third notation is optional for observation of evolution of symptoms (depending on symptoms on controls or heterogeneous behaviour).
11. Observations
- 11.1 Method.....Visual
- 11.2 Observation scale.....TMV: 0:  
Susceptibility: mosaic (aucuba in case of aucuba strain as Vi-6), growth reduction, death of plants.  
Resistance: local necrotic lesions which can lead to leave drop, systemic necrosis, vein necrosis, stem necrosis.  
PMMoV: 1-2 and PMMoV: 1-2-3:  
Susceptibility: mosaic (green), growth reduction.  
Resistance: local necrotic lesions which can lead to cotyledon drop, systemic 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] susceptible  
present ..... [9] resistant
13. Critical control points.....For TMV: 0, plants with no symptoms at all have to be interpreted as escapes of inoculation.
- Recommended dates of notation should be adapted depending of expression of symptoms on controls.

Environmental conditions can have an effect on the expression of symptoms over time. In this case a third notation could be necessary.

**Ad. 48: Resistance to *Potato Y virus (PVY)***

1. Pathogen.....*Potato Y virus*
2. Quarantine status .....no
3. Host species .....Sweet pepper, hot pepper, paprika and chili – *Capsicum annuum* L.
4. Source of inoculum .....GEVES<sup>5</sup> (FR), Naktuinbouw<sup>6</sup> (NL) or INIA<sup>7</sup> (SP)
5. Isolate .....For *PVY: 0 strain zb6* (the test protocol has been validated in a CPVO co-funded project<sup>8</sup> with this isolate/pathotype).  
*PVY* Pathotype 1  
*PVY* Pathotype 2
6. Establishment isolate identity .....genetically defined pepper controls (extract from ISF table ISF web site: [http://www.worldseed.org/cms/medias/file/TradeIssues/DiseasesResistance/Differentials/Pepper-potyviruses\\_Aug2013.pdf](http://www.worldseed.org/cms/medias/file/TradeIssues/DiseasesResistance/Differentials/Pepper-potyviruses_Aug2013.pdf) )

Variety	pvr gene present	PVY: 0	PVY: 1	PVY: 1-2
Early Cal Wonder, Yolo Wonder	pvr 0	S	S	S
PI152225	pvr 1	R	R	R
Yolo Y	pvr1 <sup>1</sup> (pvr 2 <sup>1</sup> )	R	S	S
Florida VR2	pvr1 <sup>2</sup> (pvr 2 <sup>2</sup> )	R	R	S
Florida VR4, Del Rey Bell, Agronomico 10	pvr3	R	R	R
Serrano Criollo de Morelos 334	pvr4	R	R	R

S= susceptible; R= resistant;

7. Establishment pathogenicity.....Test on susceptible plants
8. Multiplication inoculum
  - 8.1 Multiplication medium .....Regeneration of the virus on plant material before inoculum preparation.
  - 8.2 Multiplication variety .....On susceptible pepper variety, *PVY* Pathotype smay be multiplied on varieties which are selective for each particular pathotype. For *PVY: 0*, because tobacco *Nicotiana tabacum* cv. *Xanthi-nc* have large leaves and can produce a lot of inocula and have a faster multiplication, they are recommended for the multiplication.
  - 8.3 Plant stage at inoculation .....see 10.3
  - 8.4 Inoculation medium.....see 10.1
  - 8.5 Inoculation method .....see 10.4
  - 8.6 Harvest of inoculum .....Symptomatic fresh leaves
  - 8.7 Check of harvested inoculum....option: on *Nicotiana tabacum* cv. *Xanthi-nc*, check mosaic presence and local lesion absence (contamination by Tobamovirus) after 5-7 days.
  - 8.8 Shelf life/viability inoculum.....fresh > 1 day, desiccated > 1 year. Because problem of stability of *PVY: 0*, shipments are recommended to be done with fresh infected leaves
9. Format of the test
  - 9.1 Number of plants per genotype At least 20 plants.
  - 9.2 Number of replicates .....-
  - 9.3 Control varieties .....*PVY: 0*:  
Susceptible controls: Ferrari, Piquillo, Yolo Wonder  
Resistant controls: Andalus, Vidi, Yolo Y  
*PVY: 1*:  
Susceptible controls: Yolo Wonder, Yolo Y  
Resistant controls: Florida VR2  
*PVY: 1-2*:  
Susceptible controls: Florida VR2, Yolo Wonder, Yolo Y  
Resistant controls: Serrano Criollo de Morelos 334
  - 9.4 Test design .....add non inoculated plants
  - 9.5 Test facility .....Climate room or greenhouse. In case of test in greenhouse during period of low daylight, shadow should not be used.
  - 9.6 Temperature.....18-25°C
  - 9.7 Light .....12 hours or longer
  - 9.8 Season .....-

<sup>5</sup> [matref@geves.fr](mailto:matref@geves.fr)

<sup>6</sup> [resistentie@naktuinbouw.nl](mailto:resistentie@naktuinbouw.nl)

<sup>7</sup> [resistencias@inia.es](mailto:resistencias@inia.es)

<sup>8</sup> Harmores 2 CPVO project (<http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports> )

- 9.9 Special measures .....For PVY: 0, it is advised to choose Yolo Y as resistant control or to add the differentials in tests to be able to observe a possible contamination by PVY: 1 or 1-2
10. Inoculation
- 10.1 Preparation inoculum .....1 g leaf with symptoms with 4 mL PBS with carborundum (80mg) and activated carbon (80mg) or similar buffer, homogenize.
- 10.2 Quantification inoculum.....-
- 10.3 Plant stage at inoculation .....PVY: 0 cotyledons stage  
PVY: 1 and 1-2: cotyledon stage or first pointing leaf stage
- 10.4 Inoculation method.....rubbing with the virus suspension.
- 10.5 Final observations.....Three weeks post-inoculation.
11. Observations
- 11.1 Method.....Visual
- 11.2 Observation scale .....Susceptibility: mosaic (can be very light/faint), growth reduction, Vein banding and vein necrosis.  
Resistance: no symptoms.
- 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] | susceptible |
| present ..... | [9] | resistant   |
13. Critical control points.....Recommended dates of notation should be adapted depending of expression of symptoms on controls.

Source: ISF [isf@worldseed.org](mailto:isf@worldseed.org)

#### Ad. 49: Resistance to *Phytophthora capsici* (Pc)

##### Maintenance of inoculum

Inoculum and type of medium: *Phytophthora capsici* strain 101, to be cultivated on V8 juice-agar (1%) in Petri's dishes.

##### Conduct of test

Growth stage of plants: around eight-week old plants, grown in greenhouse (stage: first flower bud)  
Temperature: 22°C  
Light: 12 hours/day  
Method of inoculation: Plants are cut just below the point of first branching. A disc of mycelium of 4 mm in diameter should be used as inoculum. The disc is placed on the freshly cut stem. The top of the stem is wrapped with a piece of aluminium foil, to keep it wet. Infected plants are transferred to a growth chamber kept at 22°C.

##### Duration of test:

From sowing to inoculation: between 6 and 8 weeks  
From inoculation to scoring: first scoring: 7 days  
second scoring: 14 days  
final scoring: 21 days

Number of plants tested: 20 plants

##### Scoring:

The length of necrosis on the stem, induced by the fungus development, is recorded once a week during 3 weeks, on each plant. The aluminium foil on the top of the stem should be removed 7 days after the inoculation. The first reading should take place immediately after the removal of the aluminium foil. Subsequent scoring should be made on the 14<sup>th</sup> and 21<sup>st</sup> day counting from the day of inoculation. The distance (in mm) between the lowest point reached by the necrosis and the top of the stem should be recorded.

##### Standard varieties:

Susceptible: Yolo Wonder  
Resistant: Chistera, Favorol, Solario, Phyo 636 (given in the order of their level of resistance)

Ad. 50: Resistance to *Cucumber mosaic Virus* (CMV)

Maintenance of pathotypes

Strain: Fulton  
Type of medium: On susceptible plants: *Vinca rosea*  
Special conditions: -

Inoculum production: Crushing of 1g of fresh leaves of *Vinca rosea* in 4 ml of Phosphate buffer 0.03M pH 7 + DIECA (diethyl dithiocaremate de sodium) (1 for 1000) + 300 mg of activated carbon + 80 mg of carborundum

Execution of test:

Growth stage of plants: Young plants at the stage of developed cotyledons. First leaf non-pointing  
Number of plants: 20 plants  
Growing conditions: 22°C, 12 hours of light  
Growing method: Raising of plants in climatised room  
Method of inoculation: Mechanical rubbing of cotyledons with a virus solution, the plants are kept in darkness for 48 hours

Duration of test:

From sowing to inoculation: 12 to 13 days  
From inoculation to reading: 3 readings at 10, 15 and 21 days after inoculation

Standard varieties:

Susceptible variety: Yolo Wonder  
Tolerant (T) or resistant (R) varieties: Milord (T)  
Vania (R)

Ad. 51: Resistance to *Tomato spotted wilt virus* Pathotype 0 (TSWV: 0)

Maintenance of pathotypes:

Type of medium: on susceptible pepper plants or freezing at -70°C

Execution of test:

Growth stage of the plants: one or two leaves expanded  
Temperature: day: 20°C, night: 20°C  
Light: extra light in winter  
Growing method: glasshouse  
Inoculation medium: 0.01 M PBS buffer with 0.1% sodium sulfite freshly added  
Method of inoculation: mechanical, rubbing with carborundum on cotyledons,  
Special conditions: keep inoculum suspension cool during inoculation

Duration of test:

from sowing to inoculation: 20 days  
from inoculation to reading: 14 to 20 days

Number of plants tested: 20 plants

Remarks: beware of thrips; resistance will break down when temperature is higher than 25°C

Standard varieties

Susceptible: Bruinsma Wonder  
Resistant: Explorer, Prior

Ad. 52: Resistance to *Xanthomonas campestris* pv. *Vesicatoria* (Xcv)

Maintenance of pathotypes

Type of medium: PDA (Potato, Dextrose, Agar) medium  
Special conditions: 48 hours *Xanthomonas campestris* pv. *vesicatoria* culture. Adjusting inoculum concentration of bacteria-cellular  $10^7$ .

Execution of test

Growth stage of plants: 6th to 8th true leaves  
Temperature: 24°C night, 25°C day  
Relative humidity: 80%  
Light: 30 000 lx, day length 16 hours  
Growing method: Sowing in boxes in climate chamber or in glasshouse  
Method of inoculation: Infiltration into abaxial surface of a leaf in 13-15 mm diameter spots  
Duration of the test: 10-14 days

Number of plants tested: 15 to 30 plants

Remarks

Genetics of bacteria pathotypes and resistant genotypes:

Resistant varieties: Aladin, Camelot, ECR-20R, Kaldóm, Kalorez, Lancelot, Pasa

## 9. LITERATURE

### GENERAL INFORMATION

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## **10. TECHNICAL QUESTIONNAIRE**

The Technical Questionnaire is available on the CPVO website under the following reference:  
CPVO-TQ/076/2-Rev.2