



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

***Citrullus lanatus* (Thunb.) Matsum. et Nakai**

### **WATERMELON**

UPOV Code: CTRLS\_LAN

**Adopted on 14/04/2021**

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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 *Citrullus lanatus* (Thunb.) Matsum. et Nakai.

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/142/5 dated 29/10/2019 (<https://www.upov.int/edocs/tgdocs/en/tg142.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability.

### **1.2 Entry into Force**

The present protocol enters into force on **15.04.2021**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

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

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

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

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

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

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

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

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

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

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

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

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

## **2. MATERIAL REQUIRED**

### **2.1 Plant material requirements**

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

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

The CPVO informs the applicant that

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

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

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

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

## **3. METHOD OF EXAMINATION**

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

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

The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

### **3.2 Testing Place**

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

3.4.3 For pollination and fruit set of triploid varieties it is needed to interplant with diploid varieties in a trial layout so that the diploid pollenizers will be close to the triploid plants. The minimum percentage of diploid plants should not be less than 30%. When pollinators (e.g. bees, bumblebees) are used a slightly lower percentage of pollenizer may be required.

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

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

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

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

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

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

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

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

#### **3.6.1 Forms of variety collection**

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

#### **3.6.2 Living Plant Material**

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

#### **3.6.3 Range of the variety collection**

The living variety collection shall cover at least those 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**

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.

## **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 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.

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

#### 4.1.5 Method of observation

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

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

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

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

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

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

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

## 4.2 **Uniformity**

4.2.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP10 '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 Protocol:

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

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

4.2.4 For the assessment of uniformity of hybrids and inbred lines, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of 20 plants, 2 off-types are 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 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed 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) Ploidy (characteristic 1)
  - b) Fruit: weight (characteristic 11)
  - c) Fruit: shape in longitudinal section (characteristic 12)
  - d) Fruit: ground colour of skin (characteristic 16)
  - e) Fruit: width of stripes (characteristic 19)
  - f) Fruit: margin of stripes (characteristic 22)
  - g) Fruit: main colour of flesh (characteristic 28)
  - h) Only diploid and tetraploid varieties: Seed: length (characteristic 31)
  - i) Only diploid and tetraploid varieties: Seed: ground colour of testa (characteristic 33)
- 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.

## 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

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)-(d)	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>Ploidy</b>		
(+)			diploid	SP 4, Sugar Baby, Yamato 3	2
<b>QL</b>			triploid	Boston, TRIX 313	3
<b>G</b>			tetraploid		4
<b>2.</b>	<b>2.</b>	<b>MS/VG</b>	<b>Cotyledon: size</b>		
<b>QN</b>		<b>(a)</b>	small	Crimson Glory, Kanro, Rapid	3
			medium	Crisby, Granit, Panni, Yamato 3	5
			large	Farao, Kurobe, Royal flesh hybrid	7
<b>3.</b>	<b>3.</b>	<b>VG</b>	<b>Cotyledon: shape</b>		
(+)		<b>(a)</b>	narrow elliptic	Kahô	1
<b>QN</b>			medium elliptic	Crimson Sweet, Farao, Napsugár, Yamato 3	2
			broad elliptic	Kanro	3
<b>4.</b>	<b>4.</b>	<b>VG</b>	<b>Cotyledon: intensity of green colour</b>		
<b>QN</b>		<b>(a)</b>	light	À graine rouge à confire à chair verte, Shin Kurobe 7	1
			medium	Jenny, Yamato 3	3
			dark	Boston, Kahô, SP 4	5
<b>5.</b>	<b>5.</b>	<b>VG</b>	<b>Leaf blade: size</b>		
<b>QN</b>		<b>(b)</b>	small	SP 1, SP 4	1
			medium	Sugar Baby	3
			large	Topgun	5
<b>6.</b>	<b>6.</b>	<b>VG/MS</b>	<b>Leaf blade: ratio length/width</b>		
(+)		<b>(b)</b>	low	Kanro	1
<b>QN</b>			medium	Sugar Baby, Yamato 3	2
			high	Kurobe	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>7.</b>	<b>7.</b>	<b>VG</b>	<b>Leaf blade: colour</b>		
<b>PQ</b>		<b>(b)</b>	yellowish green	Baby Fun, Okan	1
			green	Crimson Sweet, Yamato 3	2
			greyish green	Sugar Baby	3
			bluish green	SP 4	4
<b>8.</b>	<b>8. (*)</b>	<b>VG</b>	<b>Leaf blade: degree of lobing</b>		
<b>(+)</b>		<b>(b)</b>	absent or very weak	Sunshade	1
<b>QN</b>			weak	Estrella, Karistan	3
			medium	Crimson Sweet, Crisby	5
			strong	Cadanz	7
			very strong	SP 1	9
<b>9.</b>	<b>9.</b>	<b>VG</b>	<b>Leaf blade: blistering</b>		
<b>(+)</b>		<b>(b)</b>	weak	Tabata, Estel	1
<b>QN</b>			medium	Yamato 3	2
			strong	Klondike Striped II, Sakura	3
<b>10.</b>	<b>10.</b>	<b>VG</b>	<b>Leaf blade: colour of veins</b>		
<b>QL</b>		<b>(b)</b>	green	Asahiyamato	1
			yellow	Taiyô	2
<b>11.</b>	<b>11.</b>	<b>MG/MS</b>	<b>Fruit: weight</b>		
<b>QN</b>	<b>(*)</b>	<b>(c)</b>	very low	Monaco, New Hampshire Midget	1
			very low to low	Mini, Petite Perfection	2
			low	Angela	3
			low to medium	Pasion, Sugar Baby	4
			medium	Boston	5
			medium to high	Crimson Sweet, Panonnia	6
			high	Fabiola	7
			high to very high	Jubilee	8
<b>G</b>			very high	Carolina Cross, Cobb's Gem, Florida Giant	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>12.</b>	<b>12.</b>	<b>VG</b>	<b>Fruit: shape in longitudinal section</b>		
(+)	(*)	(c)	circular	Camilla, Kanro	1
<b>QN</b>			broad elliptic	Fumin, Gray Belle, Yellow Baby, Zorba	2
			medium elliptic	Congo, Kurobe, Picnic	3
<b>G</b>			narrow elliptic	All Sweet, Charleston Gray	4
<b>13.</b>	<b>13.</b>	<b>VG</b>	<b>Fruit: depression at base</b>		
(+)		(c)	absent or very shallow		1
<b>QN</b>			shallow	Kahô, Yellow Baby	2
			medium	Triple Sweet, Yamato 3	3
			deep	À graine rouge à confire à chair verte, Kanro	4
			very deep		5
<b>14.</b>	<b>14.</b>	<b>VG</b>	<b>Fruit: shape of apical part</b>		
(+)		(c)	truncate	Cream Sinka, Kanro	1
<b>PQ</b>			truncate to rounded		2
			rounded	Glory, Sugar Baby, Toro, Yamato 3	3
			rounded to acute		4
			acute	Kahô	5
<b>15.</b>	<b>15.</b>	<b>VG</b>	<b>Fruit: depression at apex</b>		
(+)		(c)	absent or very shallow		1
<b>QN</b>			shallow	Burpee Hybrid, Kahô, Valdoria	2
			medium	Asahi Miyako, Fumin	3
			deep	Cobb's Gem	4
			very deep		5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>16.</b>	<b>16.</b> <b>(*)</b>	<b>VG</b>	<b>Fruit: ground colour of skin</b>		
<b>(+)</b>		<b>(c)</b>	yellow	Taiyô	1
<b>PQ</b>			very light green	Ipanema	2
			very light green to light green	Napsugár	3
			light green	Tigre	4
			light green to medium green	Pepsin	5
			medium green	Ovation, Talete	6
			medium green to dark green	Odem, Resistant, Sweet Marvel	7
			dark green	Sugar Baby	8
			dark green to very dark green	Augusta, Rocio	9
<b>G</b>			very dark green		10
<b>17.</b>	<b>17.</b>	<b>VG</b>	<b>Fruit: conspicuousness of veining</b>		
<b>(+)</b>		<b>(c)</b>	inconspicuous or very weakly conspicuous	Napsugár	1
<b>QN</b>			weak		2
			medium	Crimson Sweet	3
			strong	Trix Palomar	4
			very strong		5
<b>18.</b>	<b>18.</b> <b>(*)</b>	<b>VG</b>	<b>Fruit: pattern of stripes</b>		
<b>(+)</b>		<b>(c)</b>	only one coloured	Congo	1
<b>PQ</b>			one coloured and veins	Trix Palomar	2
			one coloured, veins and marbled	Boston	3
			one coloured and marbled	Jenny	4
			two coloured, veins and marbled	Crisby	5
			only veins		6

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>19.</b>	<b>19.</b> (*)	<b>VG</b>	<b>Fruit: width of stripes</b>		
(+)		(c)	very narrow	SP 4, Tiny Orchid	1
<b>QN</b>			narrow	Boston	3
			medium	Crimson Sweet	5
			broad	Sangria	7
<b>G</b>			very broad	All Sweet	9
<b>20.</b>	<b>20.</b>	<b>VG</b>	<b>Fruit: main colour of stripes</b>		
(+)		(c)	yellow		1
<b>PQ</b>			very light green		2
			light green		3
			medium green		4
			dark green		5
			very dark green		6
<b>21.</b>	<b>21.</b> (*)	<b>VG</b>	<b>Fruit: conspicuousness of stripes</b>		
(+)		(c)	inconspicuous or very weakly conspicuous	Augusta	1
<b>QN</b>			weak	Odem	2
			medium	Trix Palomar	3
			strong	Jenny	4
			very strong	À graine rouge à confire à chair verte	5
<b>22.</b>	<b>22.</b> (*)	<b>VG</b>	<b>Fruit: margin of stripes</b>		
(+)		(c)	diffuse	Crimson Glory, Crisby	1
<b>QN</b>			medium	Crimson Sweet	2
<b>G</b>			sharp	Jenny, Jubilee	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>23.</b>	<b>23.</b>	<b>VG</b>	<b>Fruit: size of insertion of peduncle</b>		
(+)		(c)	small	Charleston Gray, Sugar Bush	3
<b>QN</b>			medium	Fumin, Picnic	5
			large	Dixie Queen, Kanro	7
<b>24.</b>	<b>24.</b>	<b>VG</b>	<b>Fruit: size of pistil scar</b>		
(+)		(c)	small	Charleston Gray, Daisen	3
<b>QN</b>			medium	Yamato 3	5
			large	Kanro, Trix Palomar	7
<b>25.</b>	<b>25.</b>	<b>VG</b>	<b>Fruit: grooving</b>		
(+)		(c)	absent or very weak	Sugar Baby	1
<b>QN</b>			weak	Augusta, Kanro, Rapid	2
			medium	Asahi Miyako Hybrid, Bego	3
			strong	Marsowszky, Napsugár, Panni	4
<b>26.</b>	<b>26.</b>	<b>VG</b>	<b>Fruit: waxy layer</b>		
(+)		(c)	absent or very weak	Betica	1
<b>QN</b>			medium	Sugar Baby	3
			very strong	Red Star, Romanza	5
<b>27.</b>	<b>27. (*)</b>	<b>VG/MS</b>	<b>Fruit: thickness of pericarp</b>		
(+)		(c)	very thin	Bibo, Tiny Orchid, Luciano	1
<b>QN</b>			thin	À graine rouge à confire à chair verte, Beni-kodama, Jenny, Kahô, Kassai	3
			medium	Pannonia, Sugar Baby, Sugar Belle, Yamato 3	5
			thick	Charleston Gray, Crimson Sweet, Kurobe, Triple Sweet, Sunrise	7
			very thick	Coles Early, Kholodok	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>28.</b>	<b>28.</b> <b>(*)</b>	<b>VS</b>	<b>Fruit: main colour of flesh</b>		
<b>(+)</b>		<b>(c)</b>	white	SP 4, SP 1, Yamato Cream 3	1
<b>PQ</b>			yellow	Napsugár, Yamato Cream 1	2
			orange	Kahô, Tendersweet	3
			pink	Sadul	4
			pinkish red	Bingo, Crimson Sweet	5
			red	Asahi Miyako Hybrid, Sugar Baby, Topgun	6
<b>G</b>			dark red	Dixie Lee	7
<b>29.</b>	<b>29.</b>	<b>VG</b>	<b><u>Only triploid varieties:</u> Seed coat: size</b>		
<b>QN</b>		<b>(d)</b>	small	Petite Perfection	2
			medium	Boston, Valdoria, Sweet Sun	3
			large	Ortal, Tigre, Pasion	4
<b>30.</b>	<b>30.</b>	<b>VG</b>	<b><u>Only diploid and tetraploid varieties:</u> Fruit: number of seeds</b>		
<b>QN</b>		<b>(d)</b>	none or few	Tanenashi Kôyô	1
			medium	Miyako 3	2
			many	Fumin	3
<b>31.</b>	<b>31.</b> <b>(*)</b>	<b>VG/MS</b>	<b><u>Only diploid and tetraploid varieties:</u> Seed: length</b>		
<b>QN</b>		<b>(d)</b>	very short	Kudam	1
			short	Pannonia, Tabata	3
			medium	Sugar Baby	5
			long	Charleston Gray, Kurobe	7
<b>G</b>			very long	Malali, Wanli	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>32.</b>	<b>32.</b>	<b>VG/MS</b>	<b><u>Only diploid and tetraploid varieties:</u> Seed: ratio length/width</b>		
<b>(+)</b>		<b>(d)</b>	very low	Wanli	1
<b>QN</b>			low	Klondike	2
			medium	Early Star	3
			high	Nubia	4
			very high	Green Citron	5
<b>33.</b>	<b>33. (*)</b>	<b>VG</b>	<b><u>Only diploid and tetraploid varieties:</u> Seed: ground colour of testa</b>		
<b>(+)</b>		<b>(d)</b>	white	Sanpaku	1
<b>PQ</b>			cream	Kurobe	2
			green	À confire allongée à graine verte, Green Citron	3
			red	À graine rouge à confire à chair verte, Red Citron	4
			red brown	Kahô	5
			brown	Otome, Sugar Baby	6
<b>G</b>			black	Yamato Cream	7
<b>34.</b>	<b>34.</b>	<b>VG</b>	<b><u>Only diploid and tetraploid varieties:</u> Seed: area of over colour</b>		
<b>(+)</b>		<b>(d)</b>	absent or very small	Estela roja	1
<b>QN</b>			small	Sugar Baby	3
			medium	Crimson Sweet	5
			large	Furia	7
			very large	Starlich	9
<b>35.</b>	<b>35.</b>	<b>VG</b>	<b><u>Only diploid and tetraploid varieties:</u> Seed: patches at hilum</b>		
<b>(+)</b>		<b>(d)</b>	absent	Daisen, Kahô	1
<b>QL</b>			present	Bonanza, Frilly	9



CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
<b>36.</b>	<b>36.</b>	<b>VG</b>	<b>Time of female flowering</b>		
(+)			early	Tiny Orchid	3
QN			medium	Sugar Baby, Yamato 3	5
			late	Kurobe	7
<b>37.</b>	<b>37.</b>	<b>VG</b>	<b>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>niveum</i></b>		
(+)					
<b>37.1</b>	<b>37.1</b>		<b>Race 0</b>		
QL			absent	Kahô, Sugar Baby	1
			present	Calhoun Gray, Charleston Gray	9
<b>37.2</b>	<b>37.2</b>		<b>Race 1</b>		
QL			absent	Charleston Gray, Kahô, Sugar Baby	1
			present	Calhoun Gray	9
<b>37.3</b>	<b>37.3</b>		<b>Race 2</b>		
QL			absent	Calhoun Gray, Kahô	1
			present	PI 296341-FR	9
<b>38.</b>	<b>38.</b>	<b>VG</b>	<b>Resistance to <i>Colletotrichum orbiculare</i></b>		
(+)					
<b>38.1</b>	<b>38.1</b>		<b>Race 1</b>		
QL			absent	Black Diamond, Calhoun Gray, Kahô	1
			present	Charleston Gray, Congo, Jubilee	9

## 8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

### 8.1 Explanations covering several characteristics

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

- (a) Cotyledon: Observations on the cotyledon should be observed when the cotyledons are fully developed and before the development of the first leaf: the surface is flat and the attitude is horizontal.



- (b) Leaf blade: Observations on the leaf blade should be made on fully developed leaves on the main vine, from the 10<sup>th</sup> to the 15<sup>th</sup> leaf, during fruit set, before the fruits are developed.
- (c) Fruit: Observations on the fruit should be made on first well developed, mature fruits.
- (d) Seed: Observations on the seed should be recorded on fully developed, mature seeds harvested from the fruit.

### 8.2 Explanations for individual characteristics

#### Ad. 1: Ploidy

Ploidy level may be detected by several methods:

- by counting chromosomes of cells under the microscope;
- by counting the number chloroplasts of stomatal guard cells using a leaf peel under the microscope;
- by flow cytometry.
- Triploid varieties show a whitish seed coat without embryo.

#### Ad. 3: Cotyledon: shape



1  
narrow elliptic



2  
medium elliptic



3  
broad elliptic

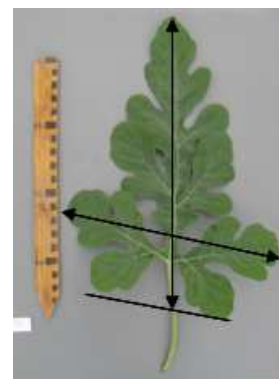
Ad. 6: Leaf blade: ratio length/width



1  
low



2  
medium



3  
high

Ad. 8: Leaf blade: degree of lobing



3  
weak



5  
medium



7  
strong

Ad. 9: Leaf blade: blistering

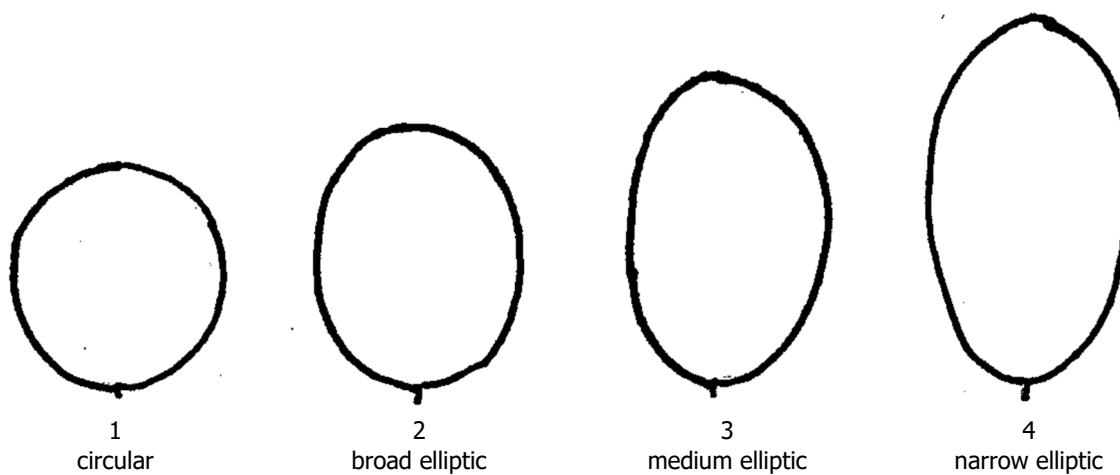


1  
weak

2  
medium

3  
strong

Ad. 12: Fruit: shape in longitudinal section



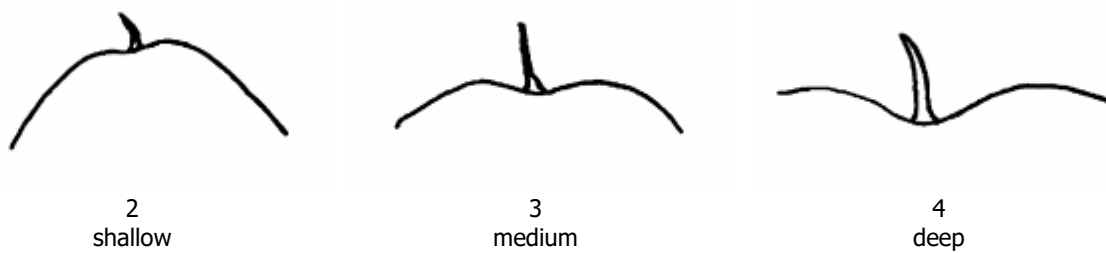
1  
circular

2  
broad elliptic

3  
medium elliptic

4  
narrow elliptic

Ad. 13: Fruit: depression at base

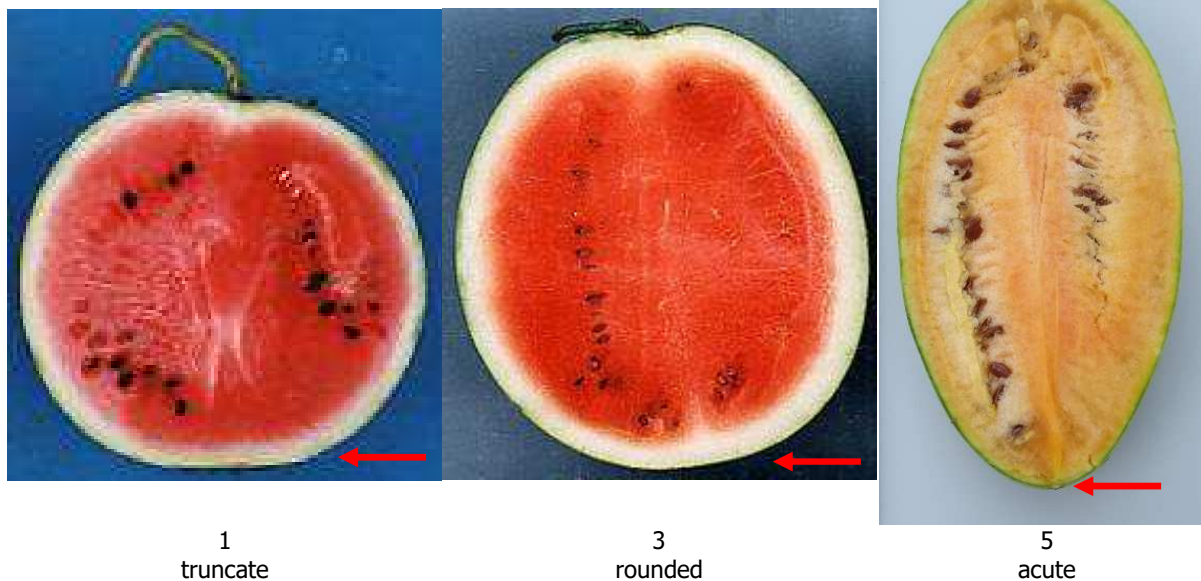


2  
shallow

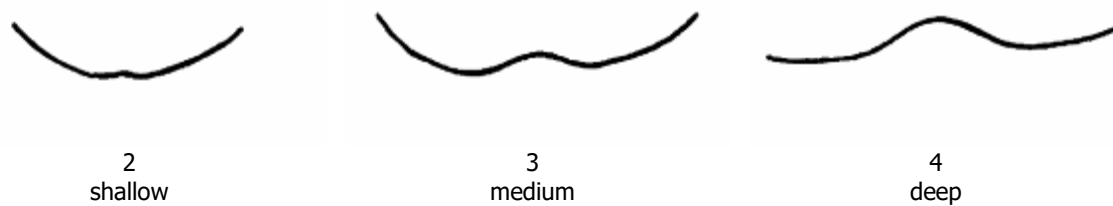
3  
medium

4  
deep

Ad. 14: Fruit: shape of apical part



Ad. 15: Fruit: depression at apex



Ad. 16: Fruit: ground colour of skin

The ground colour is the lightest colour of the skin. In case of striped fruits, the darker colour of the skin concerns the stripes.

Ad. 17: Fruit: conspicuousness of venining



1  
inconspicuous or very weakly conspicuous



2  
weak



3  
medium



4  
strong

Ad. 18: Fruit: pattern of stripes



1  
only one coloured



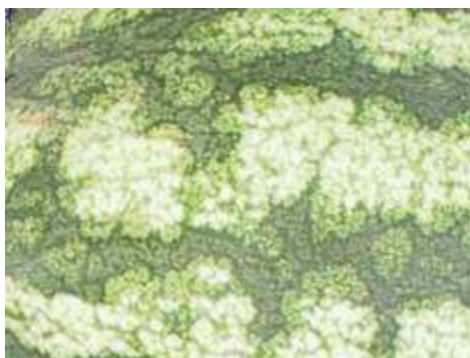
2  
one coloured and veins



3  
one coloured, veins and marbled



4  
one coloured and marbled



5  
two coloured, veins and marbled



6  
only veins

Ad. 19: Fruit: width of stripes

The ground colour is the lightest colour of the skin. In case of striped fruits, the darker colour of the skin concerns the stripes.



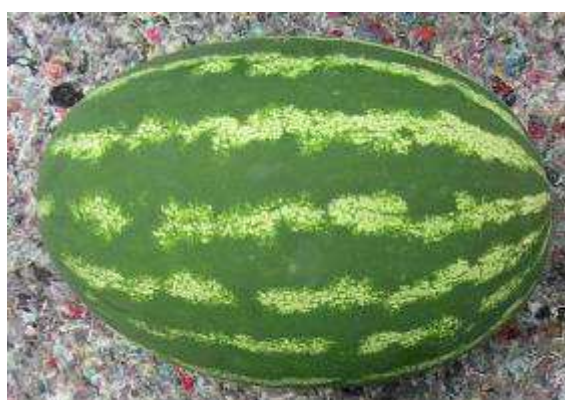
1  
very narrow



3  
Narrow



5  
medium



7  
Broad



9  
very broad

Ad. 20: Fruit: main colour of stripes

The colour of the stripes is darker than the ground colour of the skin. In case the stripes have more than one colour the one with the largest total surface area is the main color. In cases where the areas of the main and secondary color are too similar to reliably decide which color has the largest area, the darkest color is considered to be the main color.



Ad. 21: Fruit: conspicuousness of stripes



1  
inconspicuous or very weakly conspicuous



2  
Weak



3  
medium



4  
Strong



5  
very strong

Ad. 22: Fruit: margin of stripes



Ad. 23: Fruit: size of insertion of peduncle

The size of the insertion of the peduncle is absolute and not relative to fruit size.

Ad. 24: Fruit: size of pistil scar

The size of the pistil scar is absolute and not relative to fruit size

Ad. 25: Fruit: grooving



1  
absent or very weak



3  
medium

Ad. 26: Fruit: waxy layer



1  
absent or very weak



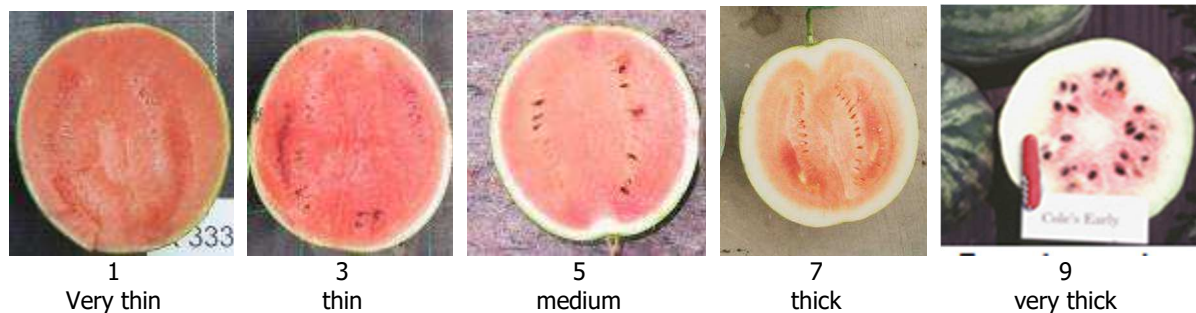
3  
medium



5  
very strong

Ad. 27: Fruit: thickness of pericarp

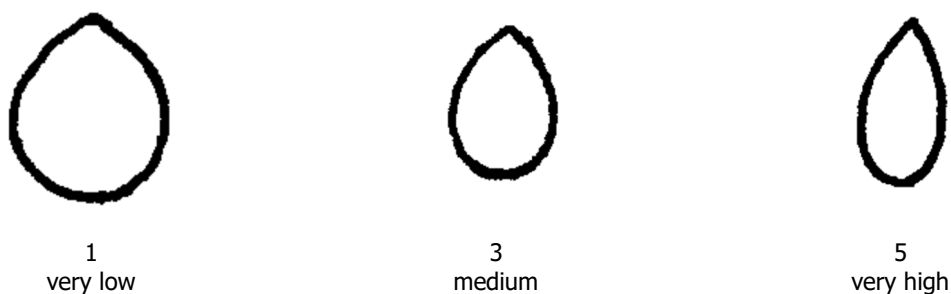
The thickness of pericarp is absolute and not relative to fruit size.



Ad. 28: Fruit: main colour of flesh

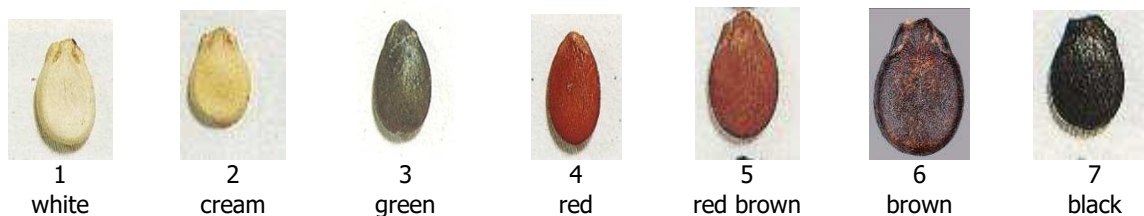
The main colour of the flesh should be observed at fruits cut in longitudinal section. In case the flesh has two colours, the one with the largest total surface area is the main colour. In cases where the areas of the main and secondary colour are too similar to reliably decide which colour has the largest area, the darkest colour is considered to be the main colour.

Ad. 32: Only diploid and tetraploid varieties: Seed: ratio length/width



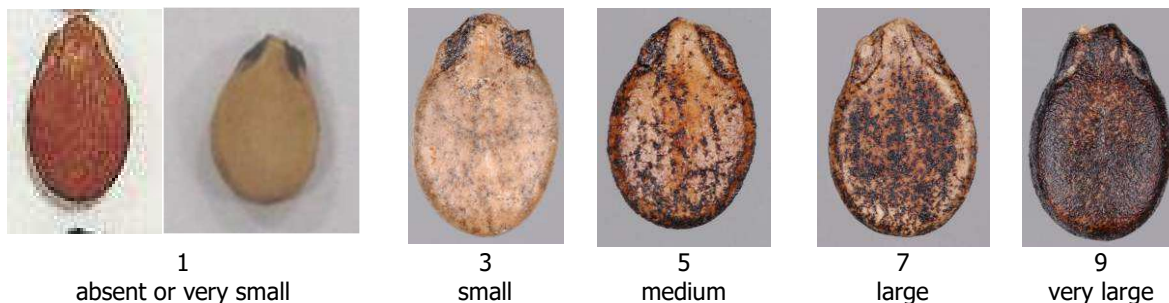
Ad. 33: Only diploid and tetraploid varieties: Seed: ground colour of testa

The ground colour is the first colour of the testa to appear during the development of the seed. The over colour is the colour that develops over time upon the ground colour, and appears as black spots.



Ad. 34: Only diploid and tetraploid varieties: Seed: area of over colour

The ground colour is the first colour of the testa to appear during the development of the seed. The over colour is the colour that develops over time upon the ground colour, and appears as black spots. The patches directly around the hilum should be excluded from the observation. Observations should be made excluding varieties with ground color black.



*Photo 1(left): Courtesy of KANDA Seed Co.*

Ad. 35: Only diploid and tetraploid varieties: Seed: patch at hilum

Observations should be made excluding with ground color black.



*Photo 1: Courtesy of KANDA Seed Co.*

Ad. 36: Time of female flowering

50% of plants with at least one female flower.

Ad. 37: Resistance to *Fusarium oxysporum* f. sp. *niveum*

1.	Pathogen .....	<i>Fusarium oxysporum</i> f. sp. <i>niveum</i>		
2.	Quarantine status .....	no		
3.	Host species.....	watermelon ( <i>Citrullus lanatus</i> )		
4.	Source of inoculum.....	Naktuinbouw		
5.	Isolate.....	Fon: 0, 1, 2		
6.	Establishment isolate identity....	differentials		
7.	Establishment pathogenicity .....	susceptible varieties		
8.	Multiplication inoculums .....	PDA or PSA; renew from frozen stock annually		
9.	Format of the test			
9.1	Number of plants per genotype.	20		
9.2	Number of replicates.....	2		
9.3	Control varieties .....	differentials		
		Fon	Fon	Fon
		: 0	: 1	: 2
		-----		
	Black Diamond, Kahô	S	S	S
	Charleston Gray	R	S	S
	Calhoun Gray	R	R	S
	P.I. 296341-FR	R	R	R
9.4	Test design .....	include at least the two most informative differential varieties		
9.5	Test facility .....	glasshouse or climate room		
9.6	Temperature .....	day 25°C, night 15°C		
9.7	Light .....	> 12 hours		
9.9	Special measures.....	apply liquid fertilizer once a week		
10.	Inoculation			
10.1	Preparation inoculum .....	Czapek Dox or PS (potato and sugar); aerated liquid culture at 28°C; filter through double muslin		
10.2	Quantification inoculum.....	count $1.3 \times 10^7$ spores per ml, dilute if necessary		
10.3	Plant stage at inoculation .....	2 <sup>nd</sup> to 3 <sup>rd</sup> leaf just expanding		
10.4	Inoculation method.....	soaking of roots and of hypocotyl axis for one minute in inoculum solution. After inoculation, transplantation of plantlets in steam-sterilized soil or perlite.		
10.5	First observation.....	7 days after inoculation		
10.6	Second observation .....	14 days after inoculation		
10.7	End of test.....	21 days after inoculation		
11.	Observations			
11.1	Method.....	visual, external		
11.2	Observation scale .....	lesions equal to or more than 2 mm in size		
11.3	Validation of test .....	standard varieties		
12.	Interpretation of data in terms of UPOV characteristic states			
		[1] lesions equal to or more than 2 mm in size		
		[9] without external symptoms		
13.	Critical control points			
	Resistant plants show no or little delayed growth but no internal or external symptoms. Vascular browning is the most reliable diagnostic symptom. Plants with external symptoms should have vascular browning, otherwise the symptom may be caused by <i>Pythium</i> .			

Ad. 38: Resistance to *Colletotrichum orbiculare*

1.	Pathogen .....	<i>Colletotrichum orbiculare</i> (anthracnose)
2.	Quarantine status .....	no
3.	Host species.....	<i>Citrullus lanatus</i> (watermelon)
4.	Source of inoculum.....	Academic research
5.	Isolate.....	Co: 1
6.	Establishment isolate identity....	on differentials
7.	Establishment pathogenicity .....	on susceptible varieties
8.	Multiplication inoculum	
8.1	Multiplication medium .....	PSA, renew from frozen stock annually
9.1	Number of plants per genotype.	20
9.2	Number of replicates.....	2
9.3	Control varieties .....	differentials: Calhoun Gray susceptible, Charleston Gray resistant

- 9.4 Test design ..... including at least the most informative differentials
- 9.5 Test facility ..... glasshouse or climate room
- 9.6 Temperature ..... day 25°C, night 16°C
- 9.7 Light ..... >12 hours
- 9.9 Special measures..... inoculated plants should be placed in a dark and humid chamber at 25°C with 100% relative humidity for 48 hours before being moved to the greenhouse.
10. Inoculation
- 10.1 Preparation inoculum ..... shaking culture in P.D. (Potato and Dextrose) liquid medium for 7 to 10 days at 28°C. Filter the medium through double muslin cloth.
- 10.2 Quantification inoculum.....  $1.5 \times 10^4$  spores per ml
- 10.3 Plant stage at inoculation ..... 2<sup>nd</sup> or 3<sup>rd</sup> leaf just expanding
- 10.4 Inoculation method..... spraying of inoculum on leaf and stem
- 10.5 First observation..... 7 days
- 10.7 End of test..... 10 days
11. Observations
- 11.1 Method..... visual
- 11.2 Observation scale ..... lesions equal to or more than 2 mm in size  
lesions may coalesce and kill the leaf back to the petiole.
- 11.3 Validation of test ..... on standard varieties
12. Interpretation of data in terms of UPOV characteristic states  
[1] lesions equal to or more than 2 mm in size  
[9] lesions absent or less than 2 mm in size
13. Critical control points  
Lesions that stay small and tend towards necrosis indicate resistance. Complete absence of symptoms indicates a low disease pressure or high resistance.

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## 10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the [CPVO website](#) under the following reference:  
CPVO-TQ/142/2-Rev - *Citrullus lanatus* (Thunb.) Matsum. et Nakai – leaf chicory