

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Citrullus lanatus (Thunb.) Matsum. et Nakai

WATERMELON

UPOV Code: CTRLS_LAN

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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/en/publications/intro_dus.htm), its associated TGP documents (http://www.upov.int/en/publications/tgp/) and the relevant UPOV Test Guideline TG/142/5 dated 20/03/2013 (http://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 **19.03.2014**. Any on-going DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

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

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

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

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

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report. If a report is negative the Examination Office shall set out the detailed reasons for its findings.

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

1.3.2 Informing on problems in the DUS test

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

1.3.3 Sample keeping in case of problems

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

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

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

2.3 Informing about problems on the submission of material

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

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

METHOD OF EXAMINATION 3.

3.1 Number of growing cycles

Two independent growing cycles

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

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

3.2 **Testing Place**

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness" http://www.upov.int/export/sites/upov/en/publications/tqp/documents/tqp 9 1.pdf.

3.3 **Conditions for Conducting the Examination**

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

3.4 Test design

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

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

3.4.3 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 Additional tests

In accordance with Article 83(3) of Council Regulation No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, an additional test may be undertaken providing that a technically acceptable test procedure can be devised.

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

3.6 Constitution and maintenance of a variety collection

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

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

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

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

3.6.1 Forms of variety collection

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

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

(b) Vegetatively propagated agricultural and vegetable species

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

3.6.2 Living Plant Material

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

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

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

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

3.6.3 Range of the variety collection

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

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

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

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

3.6.5 <u>Maintenance and renewal/update of a living variety collection</u>

(a) Seed propagated species

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

(b) Vegetatively propagated species

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

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

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

4.1 Distinctness

4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (<u>http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp 9 1.pdf</u>) prior to making decisions

regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

4.1.2. Consistent differences

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

4.1.3 Clear differences

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

4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts taken from each of 10 plants and any other observations made on all plants in the test, disregarding any off-type plants.

When resistance characteristics are used for assessing distinctness, uniformity and stability, records must be taken under conditions of controlled infection and, unless otherwise indicated, on at least 20 plants.

4.1.5 <u>Method of observation</u>

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

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'

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

(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.

(b) Hybrid varieties and inbred lines

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 a sample size 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' (<u>http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp 11 1.pdf</u>) 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 plant stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. GROUPING OF VARIETIES AND ORGANIZATION OF THE GROWING TRIAL

- **5.1** The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.
- **5.2** Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.
- **5.3** The following have been agreed as useful grouping characteristics:
 - a) 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) <u>Only diploid and tetraploid varieties</u>: Seed: length (characteristic 31)
 - i) <u>Only diploid and tetraploid varieties</u>: Seed: ground colour of testa (characteristic 33)
- **5.4** If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the examination office shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

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

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

(a) Technical Protocols allowing the use of electrophoresis

6.1.1 The list of characteristics derived from electrophoresis as in Annex X should only be used as a complement to other differences in morphological or physiological characteristics.

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

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

States of expression and corresponding notes

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

State	Note
small	3
medium	5
large	7

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

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

6.2 Example Varieties

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

6.3 Legend

For the CPVO N°	column:	
G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	 – see Chapter 6.1.2
MG, MS, VG, VS	– see Chapter 4.1.5	
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
-	-	
<i></i>		

(a)-(d) See Explanations on the Table of Characteristics in Chapter 8.1
 (+) See Explanations on the Table of Characteristics in Chapter 8.2

For the UPOV N° column:

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

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

7. TABLE OF CHARACTERISTICS

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

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

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12.	12. (*)	VG	Fruit: shape in longitudinal section		
(+)		(c)	circular	Camilla, Kanro	1
QN			broad elliptic	Fumin, Gray Belle, Yellow Baby, Zorba	2
			medium elliptic	Congo, Kurobe, Picnic	3
G			narrow elliptic	All Sweet, Charleston Gray	4
13.	13.	VG	Fruit: depression at base		
(+)		(c)	absent or very shallow		1
QN			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
14.	14.	VG	Fruit: shape of apical part		
(+)		(c)	truncate	Cream Sinka, Kanro	1
PQ			truncate to rounded		2
			rounded	Glory, Sugar Baby, Toro, Yamato3	3
			rounded to acute		4
			acute	Kahô	5
15.	15.	VG	Fruit: depression at apex		
(+)		(c)	absent or very shallow		1
QN			shallow	Burpee Hybrid, Kahô, Valdoria	2
			medium	Asahi Miyako, Fumin	3
			deep	Cobb's Gem	4
			very deep		5

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CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
16.	16. (*)	VG	Fruit: ground colour of skin		
(+)		(c)	yellow	Taiyô	1
PQ			very light green	Ipanema	2
			very light green to light green	Napsugar	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
G			very dark green		10
17.	17.	VG	Fruit: conspicuousness of veining		
(+)		(c)	inconspicuous or very weakly conspicuous	Napsugar	1
QN			weak		2
			medium	Crimson Sweet	3
			strong	Trix Palomar	4
			very strong		5
18.	18. (*)	VG	Fruit: pattern of stripes		
(+)		(c)	only one coloured	Congo	1
PQ			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
19.	19. (*)	VG	Fruit: width of stripes		
(+)		(c)	very narrow	SP 4, Tiny Orchid	1
QN			narrow	Boston	3
			medium	Crimson Sweet	5
			broad	Sangria	7
G			very broad	All Sweet	9
20.	20.	VG	Fruit: main colour of stripes		
(+)		(c)	yellow		1
PQ			very light green		2
			light green		3
			medium green		4
			dark green		5
			very dark green		6
21.	21. (*)	VG	Fruit: conspicuousness of stripes		
(+)		(c)	inconspicuous or very weakly conspicuous	Augusta	1
QN			weak	Odem	2
			medium	Trix Palomar	3
			strong	Jenny	4
			very strong	A graine rouge à confire à chair verte	5
22.	22. (*)	VG	Fruit: margin of stripes		
(+)		(c)	diffuse	Crimson Glory, Crisby	1
QN			medium	Crimson Sweet	2
G			sharp	Jenny, Jubilee	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
23.	23.	VG	Fruit: size of insertion of peduncle		
(+)		(c)	small	Charleston Gray, Sugar Bush	3
QN			medium	Fumin, Picnic	5
			large	Dixie Queen, Kanro	7
24.	24.	VG	Fruit: size of pistil scar		
(+)		(c)	small	Charleston Gray, Daisen	3
QN			medium	Yamato 3	5
			large	Kanro, Trix Palomar	7
25.	25.	VG	Fruit: grooving		
(+)		(c)	absent or very weak	Sugar Baby	1
QN			weak	Augusta, Kanro, Rapid	2
			medium	Asahi Miyako Hybrid, Bego	3
			strong	Marsowszky, Napsugár, Panni	4
26.	26.	VG	Fruit: waxy layer		
(+)		(c)	absent or very weak	Betica	1
QN			medium	Sugar Baby	3
			very strong	Red Star, Romanza	5
27.	27. (*)	VG/MS	Fruit: thickness of pericarp		
(+)		(c)	very thin	Bibo, Tiny Orchid, Luciano	1
QN			thin	À graine rouge à confire à chair verte, Beni-kodama, Jenny, Kahô, Kassai	3
			medium	Panonnia, 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
28.	28. (*)	VS	Fruit: main colour of flesh		
(+)		(c)	white	SP 4, SP 1, Yamato Cream 3	1
PQ			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
G			dark red	Dixie Lee	7
29.	29.	VG	Only triploid varieties: Seed coat: size		
QN		(d)	small	Petite Perfection	2
			medium	Boston, Valdoria, Sweet Sun	3
			large	Ortal, Tigre, Pasion	4
30.	30.	VG	<u>Only diploid and tetraploid varieties</u> : Fruit: number of seeds		
QN		(d)	none or few	Tanenashi Kôyô	1
			medium	Miyako 3	2
			many	Fumin	3
31.	31. (*)	VG/MS	<u>Only diploid and tetraploid varieties</u> : Seed: length		
QN		(d)	very short	Kudam	1
			short	Panonnia, Tabata	3
			medium	Sugar Baby	5
			long	Charleston Gray, Kurobe	7
G			very long	Malali, Wanli	9

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

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
36.	36.	VG	Only diploid and tetraploid varieties: Seed: patches at hilum		
(+)		(d)	absent or very weak	Daisen, Kahô	1
QN			medium	Kurobe, Rattle Snake, Yamato 3	2
			strong		3
37.	37.	VG	Time of female flowering		
(+)			early	Tiny Orchid	3
QN			medium	Sugar Baby, Yamato 3	5
			late	Kurobe	7
38. (+)	38.	VG	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>niveum</i>		
38.1	38.1		Race 0		
QL			absent	Kahô, Sugar Baby	1
			present	Calhoun Gray, Charleston Gray	9
38.2	38.2		Race 1		
QL			absent	Charleston Gray, Kahô, Sugar Baby	1
			present	Calhoun Gray	9
38.3	38.3		Race 2		
QL			absent	Calhoun Gray, Kahô	1
			present	PI 296341-FR	9
39. (+)	39.	VG	Resistance to Colletotrichum orbiculare		
39.1	39.1		Race 1		
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 10th to the 15th 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.

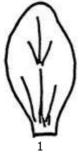
8.2 Explanations for individual characteristics

Ad. 1: Ploidy

Ploidy level may be detected by several methods:

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

Ad. 3: Cotyledon: shape



narrow elliptic



z medium elliptic



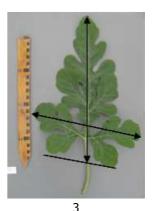
broad elliptic

Ad. 6: Leaf blade: ratio length/width





medium



high

Ad. 8: Leaf blade: degree of lobing



weak



medium



strong

Ad. 9: Leaf blade: blistering

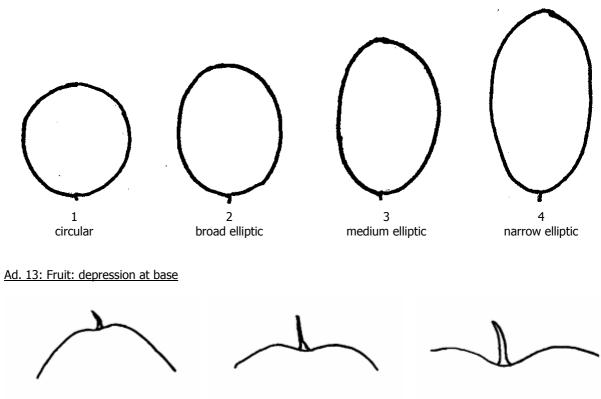


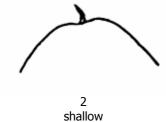
weak

medium

strong

Ad. 12: Fruit: shape in longitudinal section

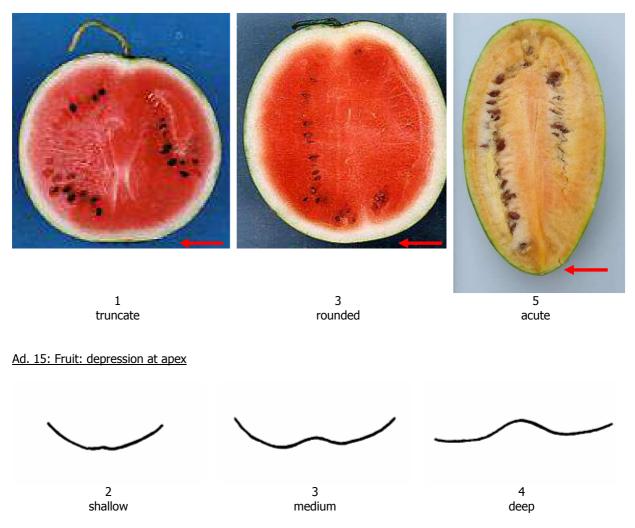




3 medium



Ad. 14: Fruit: shape of apical part



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





3 medium

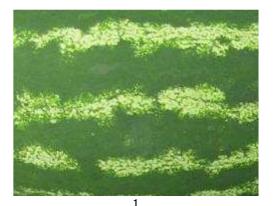


weak

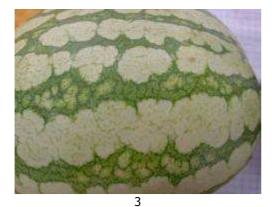


4 strong

Ad. 18: Fruit: pattern of stripes



1 only one coloured



one coloured, veins and marbled



two coloured, veins and marbled





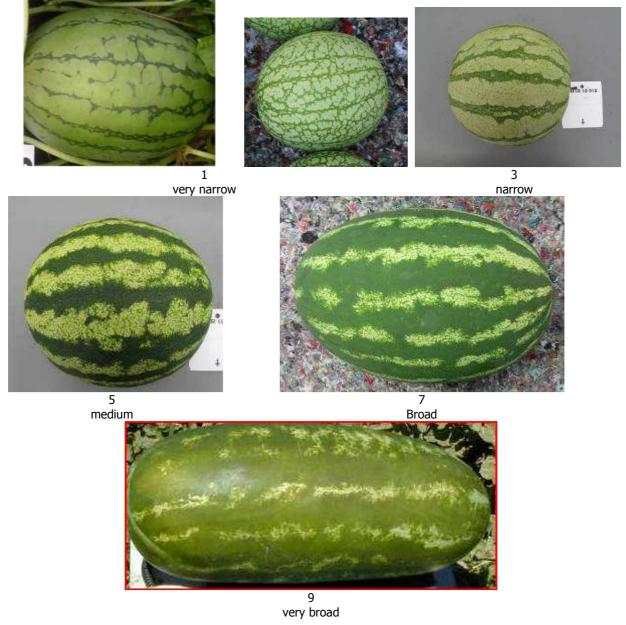
one coloured and marbled



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.



Ad. 20: Fruit: main colour of stripes

The colour of the stripes is darker than the ground color 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







3 medium

4 Strong



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



absent or very weak



medium

Ad. 26: Fruit: waxy layer



3

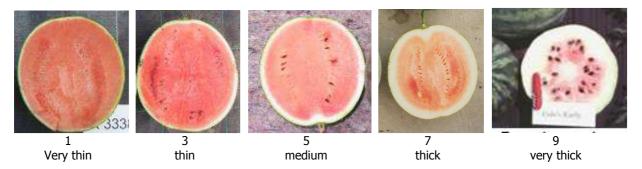




very strong

Ad. 27: Fruit: thickness of pericarp

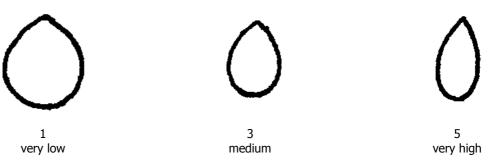
The thickness of pericarp is absolute and not relative to fruit size.



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





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.



1 white



green

3



red



5 red brown



brown

7 black

Ad. 34: Only diploid and tetraploid varieties: Seed: over 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. 35: Only diploid and tetraploid varieties: Seed: area of over colour in relation to that of ground 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.



1 very small



small



medium



7 large



9 very large

Ad. 36: Only diploid and tetraploid varieties: Seed: patch at hilum



absent or very weak

Ad. 37: Time of female flowering

50% of plants with at least one female flower.



medium



strong

Ad. 38: Resistance to Fusarium oxysporum f. sp. niveum

1. 2. 3. 4. 5. 6.	Pathogen Quarantine status Host species Source of inoculum Isolate Establishment isolate identity	<i>Fusarium oxysporum</i> f. sp <i>. niveum</i> no watermelon (<i>Citrullus lanatus</i>) Naktuinbouw Fon: 0, 1, 2 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 9.5 9.6 9.7 9.9 10.	Test design Test facility Temperature Light Special measures Inoculation	include at least the two most informative differential varieties glasshouse or climate room day 25°C, night 15°C > 12 hours apply liquid fertilizer once a week
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 1.3×10^7 spores per ml, dilute if necessary
10.3	Plant stage at inoculation	2 nd to 3 rd 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
12	Critical control points	

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 *Pythium*.

Ad. 39: Resistance to Colletotrichum orbiculare

 Pathogen Quarantine status Host species Host species	<i>Colletotrichum orbiculare</i> (anthracnose) no <i>Citrullus lanatus</i> (watermelon) Academic research Co: 1 on differentials on susceptible varieties PSA, renew from frozen stock annually 20 2
--	---

9.3 9.4 9.5 9.6 9.7 9.9	Control varieties Test design Test facility Temperature Light Special measures	differentials: Calhoun Gray susceptible, Charleston Gray resistant including at least the most informative differentials glasshouse or climate room day 25°C, night 16°C >12 hours 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	5
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*10 ⁴ spores per ml
10.3	Plant stage at inoculation	2 nd or 3 rd 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
	<u> </u>	

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.

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference: CPVO-TQ/142/2