

## PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Citrus L. - Group 3

#### **LEMONS and LIMES**

UPOV Species Code: CITRU, CITRU\_AUR, CITRU\_LAT, CITRU\_LIM

Adopted on 18/11/2004

#### I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV guideline TG/203/1 dated 09/04/2003 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies for all varieties of the following group of the genus *Citrus* L. (*Rutaceae*), and their hybrids: LEMONS AND LIMES. See below for the list of species and their subgroups:

Botanical taxon	Sub- group	Common name
Citrus assamensis S. Dutta & S.C. Bhattach.	LEM	
Citrus aurantiifolia (Christm.) Swingle	SAL	Mexican Lime
Citrus aurata Risso	LEM	
Citrus balotina Poit. & Turpin	LEM	
Citrus bergamia Risso & Poit.	SAL	
Citrus davaoensis (Wester) Tanaka	SAL	
Citrus duttae Tanaka	LEM	
Citrus excelsa Wester	SAL	
Citrus hyalopulpa Tanaka	SAL	
Citrus jambhiri Lush.	LEM (RLM)	Rough Lemon
Citrus javanica Blume	SAL	
Citrus karna Raf.	LEM	
Citrus latifolia (Yu. Tanaka) Tanaka	SAL (LAL)	Acid Lime
Citrus limetta Risso	LEM	
Citrus limettioides Tanaka	SAL (SWL)	Sweet Lime
Citrus limon (L.) Burm. f.	LEM	Lemon
Citrus limon (L.) Burm. x C. aurantifolia (Christm.) Swing.	HLL	Lemonime
Citrus limonia Osbeck	LEM	
Citrus longilimon Tanaka	LEM	
Citrus longispina Wester	SAL	
Citrus lumia Risso & Poit.	LEM	
Citrus macrolimon Tanaka	LEM	
Citrus megaloxycarpa Lush.	LEM	
Citrus mellarosa Risso	LEM	
Citrus meyeri Yu. Tanaka	LEM	
Citrus montana (Wester) Tanaka	SAL	
Citrus obversa Hassk.	SAL	
Citrus ovata Hassk.	SAL	

Botanical taxon	Sub- group	Common name
Citrus papaya Hassk.	SAL	
Citrus peretta Risso	LEM	
Citrus pseudolimon Tanaka	LEM	
Citrus pseudolimonum Wester	SAL	
Citrus pyriformis Hassk.	LEM	
Citrus rissoi Risso	LEM	
Citrus sarbati Tanaka	LEM	
Citrus webberii Wester	SAL	

### II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

# 1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the examination office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

#### 2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO whether acceptable plant material has been received or not. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

#### 3. Plant material requirements

The final dates for request for technical examination and sending of Technical Questionnaire by the CPVO as well as submission date, quantity and quality of plant material by the applicant can be found in the S2 supplement of the CPVO Official Gazette and the CPVO website (www.cpvo.europa.eu).

Quality of plants:

As regards the health status, should not be less than the standards laid down in Council Directive 77/93/EEC, 92/34/EEC, and 2000/29/EC. The plant material must be free from:

## Insects, mites and nematodes at all stages of their development

- Aleurothrixus floccosus (Mashell)
- *Meloidogyne* spp.
- Parabemisia myricae (Kuwana)
- Tylenchulus semipenetrans

#### **Fungi**

- Phytophtora spp.

#### Viruses and virus-like organisms, and in particular

- Citrus leaf rugose
- Disease that induce psorosis-like young leaves symptoms such as: psorosis, ring pot cristacortis, impietratura, concave gum.
- Infectious variegation
- Viroids such as exocortis, cachexiaxyloporosis

Chemical treatment:

The plant material must not have undergone any treatment unless the CPVO and the examination office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Labelling of individual plants in sample:

- Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination office's reference (if known)
- Name of applicant
- The phrase "On request of the CPVO"

#### III CONDUCT OF TESTS

#### 1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.

The composition of the variety collection in each Examination Office depends on the environmental conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material, Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

#### 2. <u>Material to be examined</u>

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other Offices involved in DUS testing of lemons and limes. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

#### 3. <u>Characteristics to be used</u>

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the Annex 1. 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. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N° 1239/95, to insert additional characteristics and their expression in respect of a variety.

#### 4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characters used for grouping could be the following:

- a) Young leaf: presence of anthocyanin coloration (characteristic 5)
- b) Fruit: length (characteristic 32)
- c) Fruit: presence of neck (characteristic 37)
- d) Fruit: presence of nipple (characteristic 42)
- e) Fruit surface: predominant colour(s) (characteristic 49)
- f) Parthenocarpy (characteristic 75)

#### 5. <u>Trial designs and growing conditions</u>

The minimum duration of tests (independent growing cycles) will normally include at least two satisfactory crops of fruit. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing period.

#### The test design is as follows

Each test should include 5 plants.

Unless otherwise indicated, all observations determined by measuring of counting should be made on 5 plants or 2 parts taken from each of 5 plants

Young leaf: observations on the young leaf should be made on actively growing spring flush.

<u>Leaf</u>: Observations on the leaf should be made on fully developed leaves on the middle third of the youngest spring flush branch sections not showing signs of active growth.

<u>Flower</u>: Unless otherwise indicated, observations on the flower bud and the flower should be made on the terminal flower bud and flower, at the time of full flowering of the variety.

Observations on the open flower should be made on the first day of opening.

<u>Flower bud</u>: Observations on the flower bud should be made when the petal tips are visible just before the opening of the bud.

<u>Fruit</u>: Observations on the fruit should be made at the stage of optimum ripeness. The fruit should be tested weekly and harvested as soon as this stage has been reached.

All fruits for observations should be taken from the periphery of the tree and fruit misformed as a result of clustering should not be sampled.

<u>Fruit surface and fruit rind</u>: Observations on the fruit surface and on the fruit rind should be made at the middle, between the base and apex of the fruit.

The observation on the oiliness of the fruit rind should be made, by peeling the fruit, within three to seven days after harvesting.

<u>Fruit flesh</u>: Observations on the flesh of the fruit should be made on a cross section through the middle of the fruit.

Seed: Observations on the seed should be made on the fresh seed.

#### 6. Special 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, 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.

#### 7. Standards for decisions

#### a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation No. 2100/94.

#### b) Uniformity

A candidate will be considered to be sufficiently uniform if the number of off-types does not exceed the number of plants as indicated in the table below. A population standard of 1% and an acceptance probability of 95% should be applied.

Table of maximum numbers of off-types allowed for uniformity standards.

Number of plants	off-types allowed
≤ <b>5</b>	0

#### c) Stability

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

#### IV REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two fruiting periods but in some cases three fruiting periods may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the examination office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

#### V LIAISON WITH THE APPLICANT

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 agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

The interim report as well as the final report shall be sent by the Examination Office to the CPVO.

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## ANNEXES TO FOLLOW

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nantitative characteristic
eudo-qualitative characteristic

## ANNEX II

Technical Questionnaire

## **ANNEX I**

# TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Characteristics		Examples	Note
1.	1.	Ploidy	diploid	Fino (LEM)	2
QL			triploid	Bearss (LAL)	3
			tetraploid		4
2. (+)	2.	Tree: growth habit	upright	Lisbon Frost (LEM)	1
PQ			spreading	Verna (LEM)	2
			drooping		3
3.	3.	Tree: density of spines	absent or sparse		1
QN			intermediate	Verna (LEM)	2
			dense	Lisbon Frost (LEM)	3
4.	4.	Tree: length of spines	short		3
QN			medium		5
			long		7
5.	5.	Young leaf: presence of anthocyanin coloration	absent	Feminello Zagara Bianca (LEM)	1
QL	G		present	Verna (LEM)	9
6.	6.	Young leaf: intensity of anthocyanin coloration	weak		3
QN			medium		5
			strong		7
7.	7.	Leaf blade: length (apical			
		leaflet in case of compound leaf)	short	Mexicana (SAL)	3
QN			medium	Bearss (LAL)	5
			long	Fino (LEM)	7

CPVO N°	UPOV N°	Characteristics		Examples	Note
8.	8.	Leaf blade: width (apical			
		leaflet in case of compound leaf)	narrow	Mexicana (SAL)	3
QN			medium	Bearss (LAL)	5
			broad	Fino (LEM)	7
9.	9.	Leaf blade: ratio length/width (apical leaflet in case of compound leaf)	small		3
QN			medium	Fino (LEM)	5
			large		7
10.	10.	Leaf blade: shape in cross section	straight or weakly concave		1
QN			intermediate		2
			strongly concave		3
11.	11.	Leaf blade: twisting	absent or weak	Fino (LEM)	1
QN			intermediate		2
			strong		3
12.	12.	Leaf blade: green colour	light		3
QN			medium		5
			dark		7
13.	13.	Leaf blade: undulation of margin	absent or weak	Fino (LEM)	1
QN			intermediate		2
			strong		3
14.	14.	Leaf blade: incisions of margin	absent		1
PQ			crenate		2
			dentate		3

CPVO N°	UPOV N°	Characteristics		Examples	Note
15. (+)	15.	Leaf blade: shape of apex	acuminate		1
PQ			acute	Fino (LEM)	2
10			obtuse	Tillo (EENT)	3
			rounded		4
16.	16.	Leaf blade: emargination	Tounded		
(+)	10.	at tip	absent		1
QL			present		9
17.	17.	Petiole: length	short	Mexicana (SAL)	3
QN			medium	Fino (LEM)	5
			long		7
18	18	Petiole: presence of wings	absent		1
QL			present	Fino (LEM)	9
19.	19.	Varieties with petiole wings			
		<u>present only</u> : Petiole: width of wings	narrow		3
QN			medium		5
			broad		7
20.	20.	Flower bud: presence of anthocyanin coloration	absent	Feminello Zagara Bianca (LEM)	1
QL			present	Verna (LEM)	9
21.	21.	Flower bud: intensity of anthocyanin coloration	weak		3
QN			medium		5
			strong	Verna (LEM)	7
22.	22.	Flower: diameter of calyx	small		3
QN			medium		5
			large		7
23.	23.	Flower: length of petal	short	Mexicana (SAL)	3
QN			medium	Bearss (LAL)	5
			long	Fino (LEM)	7
24.	24.	Flower: width of petal	narrow		3

CPVO N°	UPOV N°	Characteristics		Examples	Note
QN			medium		5
			broad		7
25.	25.	Flower: ratio length/width of petal	small		3
QN			medium		5
			large		7
26.	26.	Flower: length of stamens	short	Mexicana (SAL)	3
QN			medium	Bearss (LAL)	5
			long	Verna (LEM)	7
27.	27.	Flower: basal union of stamens	absent	Fino (LEM)	1
QL			present		9
28.	28.	Anther: colour	white		1
PQ			light yellow	Bearss (LAL)	2
			medium yellow	Verna (LEM)	3
29.	29.	Anther: viable pollen	absent	Bearss (LAL)	1
QL			present	Verna (LEM)	9
30.	30.	Style: length	short	Mexicana (SAL)	3
QN			medium	Lima dulce (SAL)	5
			long	Fino (LEM)	7
31.	31.	Infructescence: clustering of fruits	absent		1
QL			present		9
32.	32.	Fruit: length	short	Mexicana (SAL)	3
QN			medium	Bearss (LAL)	5
$\mathbf{G}$			long	Eureka (LEM)	7
33.	33.	Fruit: diameter	small	Mexicana (SAL)	3
QN			medium	Fino (LEM)	5
			large	Bergamoto Calabria (SAL)	7
34.	34.	Fruit: ratio length/diameter	small	Kalpi (LAL)	3

CPVO N°	UPOV N°	Characteristics		Examples	Note
QN			medium	Bearss (LAL)	5
			large	Verna (LEM)	7
35.	35.	Fruit: position of broadest part	towards stalk end		1
QN			at middle	Fino (LEM)	2
			towards distal end		3
<b>36.</b> (+)	36.	Fruit: general shape of proximal part (excluding			
		neck, collar and depression at stalk end)	flattened		1
PQ			slightly rounded		2
			strongly rounded		3
			tapered		4
<b>37.</b> (+)	37.	Fruit: presence of neck	absent	Lunario (LEM)	1
QL	G		present	Verna (LEM)	9
38.	38.	Necked varieties only: Fruit: length of neck	short	Fino (LEM)	3
QN			medium	Lisbon Frost (LEM)	5
			long	Verna (LEM)	7
<b>39.</b> (+)	39.	Only varieties without fruit neck: Fruit: presence of depression at stalk end	absent	Lunario (LEM)	1
QL		P-F	present		9
40.	40.	Only varieties without fruit	F		
	• • •	neck: Fruit: depth of depression at stalk end	shallow		3
QN			medium		5
			deep		7

CPVO N°	UPOV N°	Characteristics		Examples	Note
41. (+)	41.	Fruit: general shape of distal part (excluding nipple, bulging of navel			
		and depression at distal end)	flattened		1
QN			slightly rounded		2
			strongly rounded		3
<b>42.</b> (+)	42.	Fruit: presence of nipple	absent	Mexicana (SAL)	1
QL	$\mathbf{G}$		present	Verna (LEM)	9
43.	43.	Fruit: prominence of nipple	weak	Messina (LEM)	3
QN			medium	Fino (LEM)	5
			strong	Verna (LEM)	7
44.	44.	Fruit: diameter of stylar scar	small		3
QN			medium		5
			large		7
45.	45.	Fruit: persistence of style	none	Verna (LEM)	1
PQ			partial		2
			total	Bergamoto Melarosa (SAL)	3
46.	46.	Fruit: presence of radial grooves at distal end	absent		1
QL			present		9
47.	47.	Fruit: expression of radial grooves at distal end	weak		3
QN			medium		5
			strong		7
48.	48.	Fruit: colour variegation	absent		1
QL			present		9

CPVO N°	UPOV N°	Characteristics		Examples	Note
49.	49.	Fruit surface: predominant colour(s)	green		1
PQ			yellow green		2
			light yellow	Bearss (LAL)	3
			medium yellow	Verna (LEM)	4
G			yellow orange	Limon Meyer (LEM)	5
50.	50.	Fruit surface: glossiness	absent or very weak		1
QN			weak		3
			medium		5
			strong		7
			very strong		9
51.	51.	Fruit surface: roughness	smooth		3
QN			medium		5
			rough		7
52.	52.	Fruit surface: size of oil glands	all more or less the same size		1
PQ			larger ones interspersed by smaller ones		2
53.	54.	Fruit surface: conspicuousness of larger oil glands	weak		3
QN			medium		5
			strong		7
54.	55.	Fruit surface: presence of pitting and pebbling on oil glands	pitting and pebbling absent		1
PQ			pitting absent, pebbling present		2
			pitting present, pebbling absent		3
			pitting and pebbling present		4

CPVO N°	UPOV N°	Characteristics		Examples	Note
55.	57.	Fruit rind: thickness	thin	Mexicana (SAL)	3
QN			medium	Bearss (LAL)	5
			thick	Lisbon Frost (LEM)	7
56.	58.	Fruit rind: oiliness	dry		3
QN			medium		5
			oily		7
57.	59.	Fruit: main colour of flesh	light green		1
PQ			light yellow		2
			medium pink		3
58.	60.	Fruit: filling of core	absent or very sparse		1
QN			sparse		3
			medium		5
			dense		7
			very dense		9
59.	61.	Fruit: diameter of core	small		3
QN			medium		5
			large		7
60.	62.	Fruit: presence of rudimentary segments	absent or weak	Verna (LEM)	1
QN			intermediate		2
			strong		3
61.	63.	Fruit: number of well developed segments	few		3
QN			medium	Bearss (LAL)	5
			many	Kalpi (SAL)	7
62.	64.	Fruit: strength of segment walls	weak		3
QN			medium		5
			strong		7

CPVO N°	UPOV N°	Characteristics		Examples	Note
63.	65.	Fruit: length of juice vesicles	short		3
QN			medium		5
			long		7
64.	66.	Fruit: thickness of juice vesicles	thin		3
QN			medium		5
			thick		7
65.	68.	Fruit: coherence of juice vesicles	weak		3
QN			medium		5
			strong		7
66.	69.	Fruit: juiciness	low		3
QN			medium	Bearss (LAL)	5
			high		7
67.	70.	Fruit juice: total soluble solids	low		3
QN			medium		5
			high		7
68.	71.	Fruit juice: acidity	low	Lima dulce (SAL)	3
QN			medium	Bergamoto Melarosa (SAL)	5
			high	Lisbon Frost (LEM)	7
69.	72.	Fruit: strength of fibre	weak		3
QN			medium		5
			strong		7

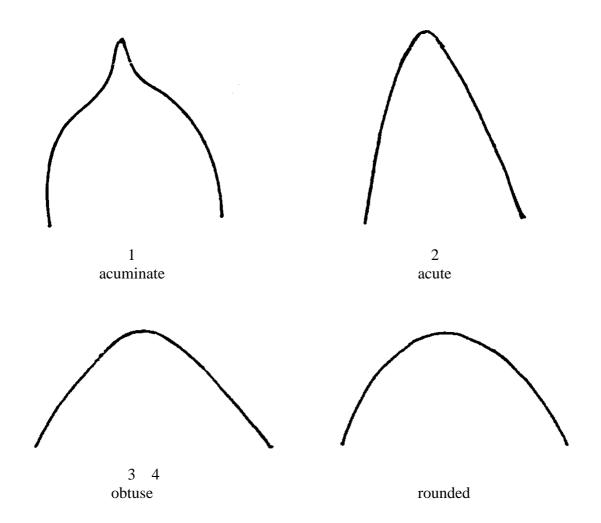
CPVO N°	UPOV N°	Characteristics		Examples	Note
<b>70.</b> (+)	73	Fruit: number of seeds (controlled manual self-			
(.)		pollination)	absent or very few	Bearss (LAL)	1
QN			few		3
			medium		5
			many		7
			very many		9
71.	74.	Fruit: number of seeds (open pollination)	absent or very few	Bearss (LAL)	1
QN			few	Verna (LEM)	3
(+)			medium	Fino (LEM)	5
			many	Kalpi (SAL)	7
72.	75.	Seed: polyembryony	absent		1
QL			present	Eureka (LEM)	9
73.	76.	Flowering habit	flowering once	Bearss (LAL)	1
QL			flowering more than once	Mexicana (SAL)	2
74.	77.	Time of maturity of fruit for consumption	early	Bearss (LAL)	3
QN			medium	Fino (LEM)	5
			late	Verna (LEM)	7
75.	78.	Parthenocarpy	absent		1
QL	G		present	Bearss (LAL)	9

## **EXPLANATIONS AND METHODS**

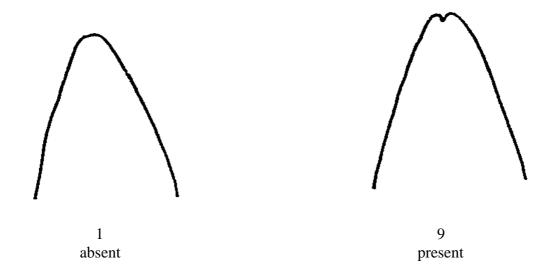
## Ad. 2: Tree: Growth habit

The observation on the growth habit of the tree should be made immediately after harvest.

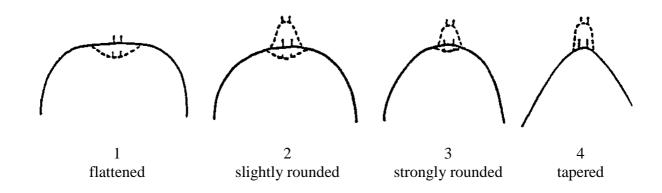
Ad. 15: Leaf blade: shape of apex



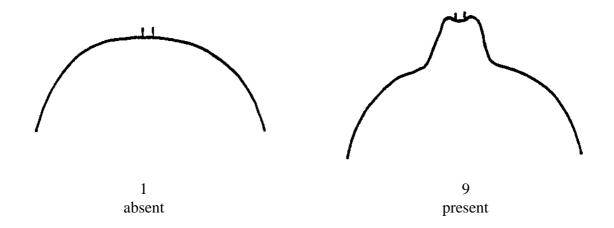
Ad. 16: Leaf blade: emargination at tip



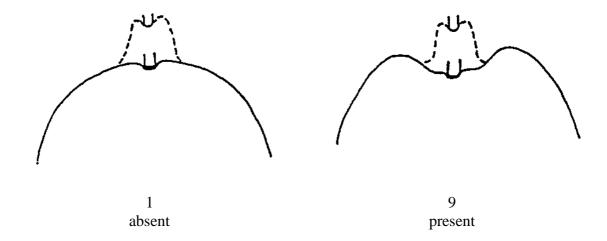
Ad. 36: Fruit: general shape of proximal part (excluding neck, collar and depression at stalk end)



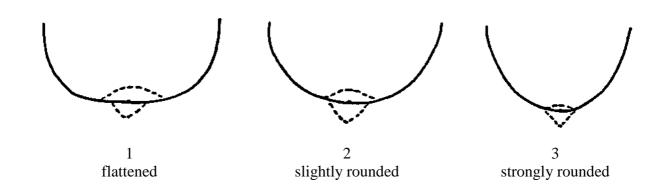
Ad. 37: Fruit: presence of neck



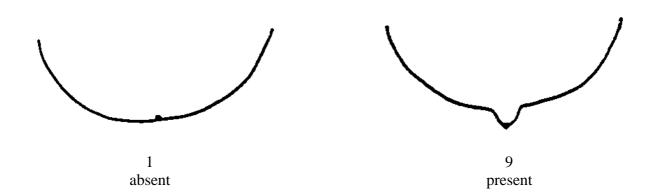
Ad. 39: Only varieties without fruit neck: Fruit: presence of depression at stalk end



Ad. 41 : Fruit:general shape of distal part (excluding nipple, bulging of navel and depression at distal end)



Ad. 42: Fruit: presence of nipple



Ad. 70: Fruit: number of seeds (controlled manual self-pollination)

Manual self-pollination is necessary to ensure a consistent production of seed.

## Ad. 71: Fruit: number of seeds (open pollination)

Open pollination means natural pollination between trees of the same variety.

## Synonym(s) of Example Variety

Example Variety	Subgroup	Synonym(s)
Bearss	LAL	Tahiti, Lima de Persia

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## **ANNEX II**

The Technical Questionnaire is available on the CPVO website under the following reference: CPVO-TQ/203/1