

# PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Citrus L. – Group 1

## **MANDARINS**

UPOV Code: CITRU, CITRU\_RET, CITRU\_UNS

Adopted on 28/10/2009

Entered into force on 09/11/2009

CPVO-TP/201/2 Draft English Date: 27/10/2009

## 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/201/1 dated 09/04/2003 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies for all varieties of the genus *Citrus* L. (Rutaceae), and their hybrids: MANDARINS. See below for the list of species and their subgroups:

GROUP 1 - ALTERNATIVE NAMES AND CORRESPONDING SUBGROUPS\*

		1
Botanical taxon	Subgroup	Common name
Citrus amblycarpa (Hassk.) Ochse	HMA	
Citrus benikoji hort. ex Tanaka	PMN	
Citrus chuana hort. ex Tseng	PMN	
Citrus clementina hort. ex Tan.	CLE	Clementine
Citrus crenatifolia Lush.	PMN	
Citrus deliciosa Ten.	MMM	Mediterranean Mandarin
Citrus depressa Hayata	HMA	
Citrus genshokan (Hayata) hort. ex Tanaka	PMN	
Citrus hainanensis Tanaka	HMA	
Citrus haniana hort. ex Tseng	PMN	
Citrus ichangensis Swing. x C. reticulata Blanco	HMR	Ichandarin
Citrus ichangensis Swing. x C. unshiu (Mak.) Marc.	HMR	Ichandarin
Citrus inflata hort. ex Tanaka	HMA	
Citrus inflatorugosa hort. ex Tanaka	HMA	
Citrus keraji hort. ex Tanaka	HMA	
Citrus leiocarpa hort. ex Tanaka	HMA	
Citrus lycopersicaeformis (Lush.) hort. ex Tanaka	HMA	
Citrus madurensis Lour.	HMA	Calamondin
Citrus maxima (Burm.) Merr. x C. ichangensis Swing.	HMR	Ichangelo
Citrus nippokoreana Tanaka	HMA	
Citrus nobilis Lour.	HMA	
Citrus oto hort. ex Yu. Tanaka	HMA	
Citrus paratangerina hort. ex Tanaka	PMN	
Citrus platymamma hort. ex Tanaka	PMN	
Citrus pseudo-aurantium hort. ex Yu. Tanaka	HMA	
Citrus pseudosunki hort. ex Tanaka	HMA	
Citrus reshni hort. ex Tanaka	HMA	
Citrus reticulata Blanco	PMN	Tangerine
Citrus reticulata Blanco x C. paradisi Macfad	TNL	Tangelo
Citrus reticulata Blanco x C. sinensis (L.) Osb.	TNR	Tangor
Citrus reticulata Blanco x Fortunella sp.	HMR	Kumandarin

These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Web Site (www.upov.int), for the latest information.]

Subgroup	Common name
PMN	
PMN	
PMN	
HMA	
PMN	
PMN	
HMA	
HMA	
НМА	Siamelo
TNR	
HMA	
SAT	Satsuma
HMA	
HMA	
HMA	Tangelolo
HMA	Tangorgelo
	PMN PMN PMN PMN PMN PMN PMN PMN HMA

### 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. <u>Final dates for receipt of documentation and material by the Examination Office</u>

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:

Should not be less than the standards laid down in Council Directive 2000/29/EC and its amendments concerning quarantine organisms, and Council Directive 2008/90/EC and Commission Directive 93/48/EEC and their amendments concerning organisms impairing quality, at the date of adoption of this protocol; please refer to "Eur-Lex" for the full text and in case of any subsequent amendments to the three aforesaid Directives.

Healthy plant material of the candidate variety should be delivered to the test station in accordance with the requirements outlined in the instructions sent by the CPVO for the submission of plant material, and which can also be consulted in the relevant entries for strawberry within the S2 Gazette and the CPVO website. In particular with respect to the phytosanitary requirements, the plant material must be accompanied by a valid certificate from a recognised authority attesting to the fact that the plant material sent for the DUS technical examination has shown negative laboratory test results for the list of pests and pathogens outlined in the pertinent entry of the examination office in the S2 Gazette/CPVO website, where the candidate mandarin variety is to undergo its DUS technical examination.

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..... - Species

- plants in sample: .....- 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"

#### ш **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 grapevine. 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. Characteristics to be used

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  $N^{\circ}$  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) Fruit: length (characteristic 20)
- b) Fruit: diameter (characteristic 21)
- c) Fruit: presence of neck (characteristic 26)
- d) Fruit surface: predominant colour(s) (characteristic 39)
- e) Time of maturity of fruit for consumption (characteristic 76)
- f) Parthenocarpy (characteristic 77)
- g) Self-incompatibility (characteristic 78)

### 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 or counting should be made on 5 plants or 2 parts taken from each of 5 plants.

#### 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
≤ 5	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.

### VI <u>ENTRY INTO FORCE</u>

The present protocol enters into force on **09/11/2009**. Any ongoing DUS examination of candidate varieties with observations started before the aforesaid date will not be affected by the approval of the new TP. Technical examinations of candidate varieties are carried out according to the TP in force the first observations are made on characteristics in an independent growing cycle.

In cases where the CPVO requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process of being carried out at the moment of the 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.

\*\*\*\*\*\*

# **ANNEXES TO FOLLOW**

ANNEX I PAGE
Table of characteristics to be used in DUS-test and preparation of descriptions
Legend:
<ul> <li>(+) See Explanations on the Table of characteristics</li> <li>(a) - (f) See Explanations on the Table of Characteristics</li> <li>G Grouping characteristics</li> </ul>
Types of expression of characteristics:
QL – Qualitative characteristic QN – Quantitative characteristic PQ – Pseudo-qualitative characteristic
Explanations and methods
Synonyms of example varieties
Literature

## ANNEX II

Technical Questionnaire

ANNEX I

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

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
1.	1.		Ploidy		
QL			diploid	Clemenules (CLE)	2
			triploid	Winola (HMA)	3
			tetraploid		4
2.	2.		Tree: growth habit		
(+)			upright	Marisol (CLE)	1
PQ			spreading	Clemenules (CLE)	2
			drooping	Owari (SAT)	3
3.	3.		Tree: density of spines		
QN			absent	Owari (SAT)	1
			sparse	Okitsu (SAT)	2
			intermediate	Marisol (CLE)	3
			dense		4
4.	4.		Tree: length of spines		
QN			short	Marisol (CLE)	3
			medium		5
			long		7
5.	5.		Leaf blade: length (apical leaflet in case of compound leaf)		
QN		(a)	short	Común (MMN)	3
			medium	Nova (HMA)	5
			long	Kara (HMA)	7
6.	6.		Leaf blade: width (apical leaflet in case of compound leaf)		
QN		(a)	narrow	Común (MMN)	3
			medium	Clemenules (CLE)	5
			broad	Page (HMA)	7

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
7.	7.		Leaf blade: ratio length/width (apical leaflet in case of compound leaf)		
QN		(a)	small	Orlando (TNL)	3
			medium	Fino (CLE)	5
			large	Clemenules (CLE)	7
8.	8.		Leaf blade: shape in cross section		
QN		(a)	straight or weakly concave	Owari (SAT)	1
			intermediate	Mineola (TNL)	2
			strongly concave	Primosole (HMA)	3
9.	13.		Leaf blade: incision of margin		
PQ		(a)	absent	Owari (SAT)	1
			crenate	Mandarino Cleopatra (MCL)	2
			dentate		3
10.	14.		Leaf blade: shape of apex		
(+)		(a)	acuminate		1
PQ			acute	Clemenules (CLE)	2
			obtuse	Mineola (TNL)	3
			rounded		4
11.	16.		Petiole: length		
QN		(a)	short	Clemenules (CLE)	3
			medium	Fortune (HMA)	5
			long	Minneola (TNL)	7
12.	17.		Petiole: presence of wings		
QL		(a)	absent	Clemenules (CLE)	1
			present	Minneola (TNL)	9
13.	20.		Flower: length of petal		
QN		(b)	short	Clementina Fina (CLE)	3
			medium	Ellendale(TNR)	5
			long	Owari (SAT)	7

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
14.	21.		Flower: width of petal		
QN		(b)	narrow	Clemenules (CLE)	3
			medium	Ellendale (TNR)	5
			broad	Owari (SAT)	7
15.	22.		Flower: ratio length/width of petal		
QN		(b)	short	Wilking (HMA)	3
			medium	Clementina Fina (CLE)	5
			large	Page (HMA)	7
16.	23.		Flower: length of stamens		
QN		(b)	short	Encore (HMA)	3
			medium	Clemenules (CLE)	5
			long	Owari (SAT)	7
17.	24.		Anther: colour		
PQ		(b)	white		1
			light yellow	Owari (SAT)	2
			medium yellow	Clementina Fina (CLE)	3
18.	25.		Anther: viable pollen		
(+)		(b)	absent or very few	Owari (SAT)	1
QN			medium	Marisol (CLE)	2
			many	Fortune (HMA)	3
19.	26.		Style: length		
QN		(b)	short	Pixie (HMA)	3
			medium	Clementina Fina (CLE)	5
			long	Owari (SAT)	7
20.	28.		Fruit: length		
QN		(c)	short	Wilking (HMA)	3
			medium	Clemenules (CLE)	5
G			long	Minneola (TNL)	7

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
21.	29.		Fruit: diameter		
QN		(c)	small	Clementina Fina (CLE)	3
			medium	Clemenules (CLE)	5
G			large	Ortanique (TNR)	7
22.	30.		Fruit: ratio length/diameter		
QN		(c)	small	Encore (HMA)	3
			medium	Clemenules (CLE)	5
			large	Minneola (TNL)	7
23.	31.		Fruit: position of broadest part		
QN		(c)	towards stalk end		1
			at middle	Clemenules (CLE)	2
			towards distal end		3
24.	32.		Fruit: shape in transverse section		
(+)		(c)	circular	Ortanique (TNR)	1
PQ			somewhat angular	Clemenules (CLE)	2
			scalloped		3
25.			Fruit: general shape of proximal part (excluding neck, collar and depression at stalk end)		
(+)		(c)	flattened	Clemenules (CLE)	1
PQ			slightly rounded	Ortanique (TNR)	2
			strongly rounded		3
			tapered		4
26.	34.		Fruit: presence of neck		
(+)		(c)	absent	Clemenules (CLE)	1
QL	G		present		9
27.	37.		Only varieties without fruit neck: Fruit: presence of depression at stalk end		
(+)		(c)	absent	Ortanique (TNR)	1
QL			present	Marisol (CLE)	9

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
28.	41.		Fruit: number of radial grooves at stalk end		
QN		(c)	absent or very few	Nova (HMA)	1
			intermediate	Clemenules (CLE)	2
			many		3
29.	44.		Fruit: presence of collar		
(+)		(c)	absent	Clemenules (CLE	1
QL			present		9
30.	48.		Fruit: general shape of distal part (excluding nipple, bulging or navel and depression at distal end)		
(+)		(c)	flattened	Clemenules (CLE)	1
QN			slightly rounded		2
			strongly rounded		3
31.	49.		Fruit: presence of depression at distal end		
(+)		(c)	absent	Ortanique (TNR)	1
QL			present	Arrufatina (CLE)	9
32.	52.		Fruit: presence of areola		
QL		(c)	absent	Nova (HMA)	1
			incomplete	Hernandina (CLE)	2
			complete	Ortanique (TNR)	3
33.	53.		Fruit: type of areola		
(+)		(c)	smooth	Owari (SAT)	1
QL			grooved		2
			ridged		3
34.	54.		Fruit: diameter of areola		
QN		(c)	small	Arrufatina (CLE)	3
			medium	Owari (SAT)	5
			large	Ortanique (TNR)	7

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
35.	55.		Fruit: diameter of stylar scar		
QN		(c)	small	Clemenules (CLE)	3
			medium	Owari (SAT)	5
			large		7
36.	56.		Fruit: persistence of style		
PQ		(c)	none	Clemenules (CLE)	1
			partial		2
			total		3
37.	57.		Fruit: presence of navel opening		
PQ		(c)	absent	Clemenules (CLE)	1
			occasionally present	Fortune (HMA)	2
			always present		3
38.	59.		Fruit: presence of radial grooves at distal end		
QL		(c)	absent	Clemenules (CLE)	1
			present		9
39.	61.		Fruit surface: predominant colour(s)		
PQ		(d)	green		1
			yellow green		2
			light yellow		3
			medium yellow	Mapo (TNL)	4
			yellow orange		5
			medium orange	Clemenules (CLE)	6
			dark orange		7
			orange red	Nova (HMA)	8
G			red		9

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
40.	62.		Fruit surface: glossiness		
QN		(d)	absent or very weak	Owari (SAT)	1
			weak	Clemenules (CLE)	3
			medium	Okitsu (SAT)	5
			strong	Nadorcott (TNR)	7
G			very strong		9
41.	63.		Fruit surface: roughness		
QN		(d)	smooth	Murcott (TNR)	3
			medium	Clemenules (CLE)	5
			rough	Temple (HMA)	7
42.	64.		Fruit surface: size of glands		
PQ		(d)	all more or less the same size		1
			larger ones interspersed by smaller ones		2
43.	67.		Fruit surface: presence of pitting and pebbling on oil glands		
PQ		(d)	pitting and pebbling absent	Nova (HMA)	1
			pitting absent, pebbling present	Loretina (CLE)	2
			pitting present, pebbling absent	Owari (SAT)	3
			pitting and pebbling present		4
44.	71.		Fruit rind: thickness		
QN		(d)	thin	Murcott (TNR)	3
			medium	Clemenules (CLE)	5
			thick	Minneola (TNL)	7
45.	72.		Fruit rind: adherence of flesh		
QN		(d)	weak	Clemenules (CLE)	3
			medium	Fortune (HMA)	5
			strong	Ortanique (TNR)	7

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
46.	73.		Fruit rind: strength		
QN		(d)	weak		3
			medium	Clemenules (CLE)	5
			strong		7
47.	74.		Fruit rind: oiliness		
QN		(d)	dry		3
			medium	Clemenules (CLE)	5
			oily	Ortanique (TNR)	7
48.	76.		Fruit: colour of albedo		
PQ		(c)	greenish		1
			white	Clemenules (CLE)	2
			light yellow	Murcott (TNR)	3
			light orange	Nadorcott (TNR)	4
			pink		5
			reddish		6
49.	77.		Fruit: density of albedo		
QN		(c)	loose	Clemenules (CLE)	3
			medium	Fortune (HMA)	5
			dense	Ortanique (TNR)	7
50.	78.		Fruit: amount of albedo adhering to flesh (strands excluded)		
QN		(c)	absent or very small	Clemenules (CLE)	1
			small		3
			medium		5
			large		7
			very large		9
51.	79.		Fruit: presence of albedo strands		
QL		(c)	absent		1
			present	Clemenules (CLE)	9

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
52.	80.		Fruit: amount of albedo strands		
QN		(c)	small		3
			medium		5
			large		7
53.	81.		Fruit: main colour of flesh		
PQ		(e)	whitish		1
			light green		2
			light yellow		3
			medium yellow	Mapo (HMA)	4
			light orange		5
			medium orange	Clemenules (CLE)	6
			dark orange		7
			red		8
			purple		9
54.	82.		Fruit: filling of core		
QN		(e)	absent or very sparse	Fortune (HMA)	1
			sparse		3
			medium	Clemenules (CLE)	5
			dense	Murcott (TNR)	7
			very dense		9
55.	83.		Fruit: diameter of core		
QN		(e)	small	Murcott (TNR)	3
			medium	Clemenules (CLE)	5
			large	Hermandina (CLE)	7
56.	84.		Fruit: presence of rudimentary segments		
QN		(e)	absent or weak	Clemenules (CLE)	1
			intermediate		2
			strong		3

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
<b>57</b> .	85.		Fruit: number of well developed segments		
QN		(e)	few	Oroval (CLE)	3
			medium	Ortanique (TNR)	5
			many	Temple (HMA)	7
58.	86.		Fruit: coherence of adjacent segment walls		
QN		(e)	weak	Clemenules (CLE)	3
			medium	Fortune (HMA)	5
			strong		7
59.	87.		Fruit: strength of segment walls		
QN		(e)	weak	Mapo (TNL)	3
			medium	Clementina Fina (CLE)	5
			strong	Oronules (CLE)	7
60.	88.		Fruit: length of juice vesicles		
QN		(e)	short	Wilking (HMA)	3
			medium		5
			long	Clemenules (CLE)	7
61.	89.		Fruit: thickness of juice vesicles		
QN		(e)	thin	Clemenules (CLE)	3
			medium		5
			thick	Mapo (TNL)	7
62.	92.		Fruit: presence of navel (viewed internally)		
PQ		(c)	absent or very rare	Clemenules (CLE)	1
			occasionally present	Nova (HMA)	2
			always present		3

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
63.	94.		Fruit: juiciness		
QN		(c)	low		3
			medium	Campeona (HMA)	5
			high	Marisol (CLE)	7
64.	95.		Fruit juice: total soluble solids		
QN		(c)	low	Okitsu (SAT)	3
			medium	Temple (HMA)	5
			high	Honey (HMA)	7
65.	96.		Fruit juice: acidity		
QN		(c)	low	Hermandina (CLE)	3
			medium	Clemenules (CLE)	5
			high	Fortune (HMA)	7
66.	97.		Fruit: strength of fibre		
QN		(c)	weak	Mapo (HMA)	3
			medium	Clemenules (CLE)	5
			strong		7
67.	98.		Fruit: number of seeds (controlled manual self-pollination)		
(+)		(f)	absent or very few	Clemenules (CLE)	1
QN			few		3
			medium	Kara (HMA)	5
			many		7
			very many	Común (MMN)	9
68			Fruit: number of seeds (controlled manual cross-pollination)		
(+)		(f)	absent or very few	Nulessin (CLE)	1
QN			few		3
			medium	Marisol (CLE)	5
			many		7
			very many	Clemenules (CLE)	9

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
69.	100.		Seed: polyembryony		
QL		(f)	absent	Wilking (HMA)	1
			present	Común (MMN)	9
70.	101.		Seed: length		
QN		(f)	short	Temple (HMA)	3
			medium		5
			long	Campeona (HMA)	7
71.	102.		Seed: width		
QN		(f)	narrow	Temple (HMA)	3
			medium		5
			broad	Campeona (HMA)	7
<b>72</b> .	103.		Seed: surface		
QL		(f)	smooth	Común (MMN)	1
			wrinkled		2
73.	105.		Seed: external colour		
PQ		(f)	greenish	Kara (HMA)	1
			whitish		2
			yellowish		3
			pinkish		4
			brownish		5
74.	106.		Seed: colour of inner seed coat		
PQ		(f)	white		1
			light yellow		2
			light brown	Murcott (TNR)	3
			medium brown		4
			dark brown		5
			red		
			purple		

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
75.	107.		Only varieties with seed: polyembrony present: Seed: colour of cotyledons		
PQ		(f)	white	Murcott (TNR)	1
			cream	Kara (HMA)	2
			light	Común (MMN)	3
			dark green		4
76.	108.		Time of maturity of fruit for consumption		
QN		(c)	early	Okitsu (SAT)	3
			medium	Clemenules (CLE)	5
G			late	Murcott (TNR)	7
77.	109.		Parthenocarpy		
QL			absent	Wilking (HMA)	1
G			present	Clemenules (CLE)	9
78.	110.		Self-incompatibility		
(+)			absent	Común (MMN)	1
QL	G		present	Clemenules (CLE)	9

#### **EXPLANATIONS AND METHODS**

Explanations covering several characteristics

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

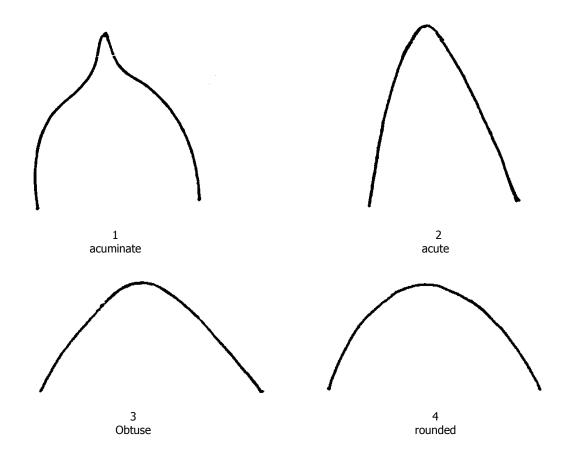
- (a) <u>Leaf</u>: Observations on the leaf should be made on fully developed leaves on the middle third of the youngest spring flush branch sections not showing signs of active growth.
- (b) Flower: Unless otherwise indicated, observations on the flower bud and the flower should be made on the terminal 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.
- (c) Fruit: 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.
- (d) Fruit surface and fruit rind: 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.
- (e) <u>Fruit flesh</u>: Observations on the flesh of the fruit should be made on a cross section through the middle of the fruit.
- (f) <u>Seed</u>: Observations on the seed should be made on the fresh seed.

#### Ad. 2: Tree: growth habit

The observation on the growth habit of the tree 7should be made immediately after harvest.

Ad. 10: Leaf blade: shape of apex

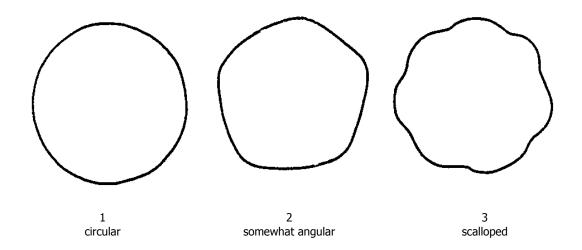


### Ad. 18: Anther: viable pollen

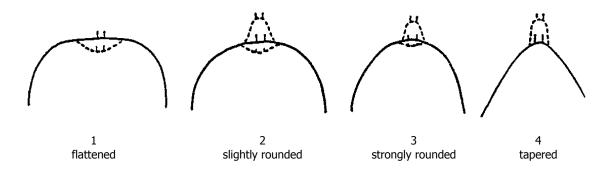
The flowers must be collected when the petals begin opening but when anthers are closed. The anthers are introduced into a Petri dish and placed inside the silica gel dryer at room temperature, during 20-48 hours of darkness. When the anthers are opened they are placed during an hour into a 8 °C chamber with a 70-80 % Relative Humidity. Afterwards, the pollen must be brushed onto a microscope slide with 2 ml of Brewbacker medium (Brewbaker and Kwack. 1963). Finally the microscope slide must be put during 20 hours into a 24 °C chamber with a 75 % RH.

The percentage of pollen fertilization is calculated by obtaining the average of pollen grains germinated observed with a binocular in 15 visual fields from 2 different microscope slides.

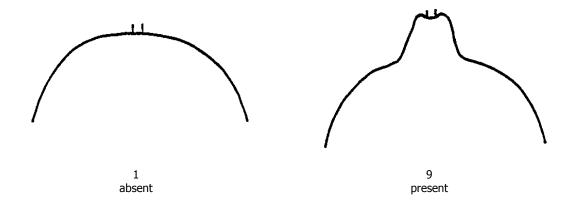
Ad. 24: Fruit: shape in transverse section



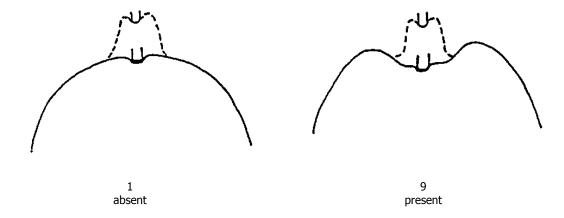
Ad. 25: Fruit: general shape of proximal part (excluding neck, collar and depression at stalk end)



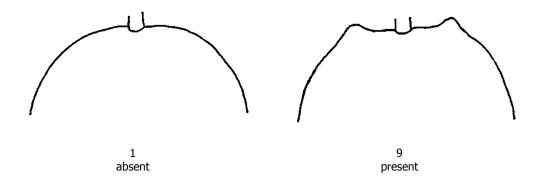
Ad. 26: Fruit: presence of neck



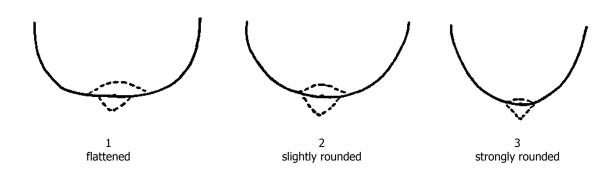
Ad. 27: Only varieties without neck: Fruit: presence of depression at stalk end



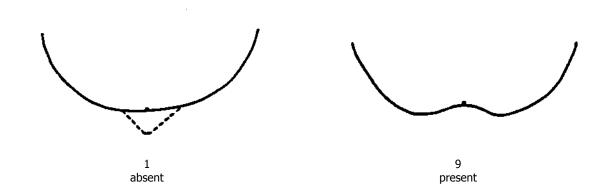
Ad. 29: Fruit: presence of collar



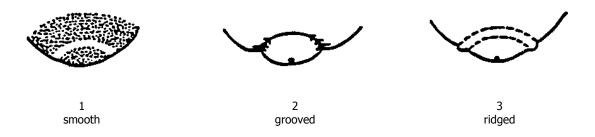
Ad. 30: Fruit: general shape of distal part (excluding nipple, bulging and depression at distal end)



Ad. 31: Fruit: presence of depression at distal end



Ad. 33: Fruit: type of areola



Ad. 67: Fruit: number of seeds (controlled manual self-pollination)

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

## Ad. 68: Fruit: number of seeds (controlled manual cross-pollination)

Pollen from a reference variety with a high fertilisation capability (e.g. Fortune) is used to pollinate manually at least 10 flowers per tree of the candidate variety.

#### Ad. 77: Self-incompatibility

A variety is self-incompatible when the fertile pollen of its own flower or of other flowers of the same variety is not able to fertilize the ovary.

The test on self-incompatability has to be carried out on at least 10 flowers.

Choose flowers with petals which are just before opening and open the flower manually. Then separate and cut the anthers. Take viable pollen from other flowers of the same variety and put it on the stigma. Cover the flowers with muslin in order to avoid accidental pollination by other pollen. If the mature fruit bears no seeds, the variety is self-incompatible. If the mature fruit bears seeds, the variety is self-compatible.

# Synonym(s) of Example Varieties

Example varieties	Subgroup	Observations	Synonym(s)
Clemenules	CLE		Clementina de Nules
Comun	MMN		Avana, Mediterranea, Wilowleaf
Clementina Fina	CLE		Fino
Minneola	TNL	Citrus paradisi Macfad. x C. tangerina hort. ex Tan. Grapefruit Duncan x Mandarin Dancy	Honeybell
Nadorcott	TNR		Afourer, Murcott Afourer
Nova	НМА	Citrus clementina hort. ex Tan. x Tangelo Orlando	Clemenvilla
Orlando	TNL	Citrus paradisi Macfad. x C. tangerina hort. ex Tan. Grapefruit Duncan x Mandarin Dancy	Lake Tangelo

### **LITERATURE**

Alexander, D. McE., 1983: "Some citrus species and varieties in Australia," Commonwealth Scientific and Industrial Research Organization, Australia, 64 pp.

Blondel, L., 1978: Botanical classification of species of the genus Citrus, Fruits 33 (11): pp. 695 - 720.

Bono, R., Soler, J., Fernandez de Cordova, L., 1986: "Variedades de agrios cultivadas en España". Generalitat Valenciana, 70 pp.

Damigella, P., Tribulato, E., Calabrese, F., Crescimanno, F.G., Continella, G., 1980: "Gli Agrumi," Cultivar. R.E.D.A., Roma, Italy, pp. 9 - 70.

Ortiz Marcide, J.M., 1985: "Nomenclatura botánica de los cítricos". Levante Agrícola nº 259-260, pp. 71-79.

Reuther, W., Webber, H.J., Batchelor, L.D. (Editors), 1967: "The Citrus Industry," Volume I, University of California, Division of Agricultural Sciences, 611 pp.

Soler, J., 1999: Reconocimiento de variedades de cítricos en campo. Generalitat Valenciana. 187 pp.

Saunt, J., 1990: "Citrus varieties of the world: an illustrated guide," Sinclair International Ltd., Norwich, England, 126 pp.

Spina, P., Russo, F., Geraci, G., Martelli, S., 1980: "Schede per il registro varietale dei fruttiferi I - ARANCIO e MANDARINO," Ministèro Agricoltura e Foreste - S.O.I., Roma, Italy, 92 pp.

Tanaka, T., 1932: "A Monograph of the Satsuma orange with special reference to the occurrence of new varieties through bud variation," reprinted from the "Memoirs of the Faculty of Science and Agriculture, Taihoku Imperial University," Volume IV, Taihoku, Formosa, Japan, 626 pp.

Zaragoza, S., Navarro, L., Cebolla, V., 1997: "Evaluation of the field collection through the germo data-base". Proceedings of the sectorial meeting of the mediterranean citrus net work (Mecinet) on global cooperation for citrus germplasm conservation and use, pp. 142-148.

Zaragoza, S., Trenor, I., Alonso, E., Medina, A., Pina, J.A., Navarro, L., 1995: "Evaluación de la colección de variedades del Banco de Germoplasma de Cítricos del IVIA: Planteamiento y primeros resultados generales". Levante Agrícola nº 331, pp. 145-149.

# **ANNEX II**



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

4.	Information on origin, maintenance and reproduction of the variety							
4.1			maintenance and reproduction of the variety cate breeding scheme, parents and other relevant information					
	Varie	Variety resulting from:						
	(a)	Cros (i)	ssing controlled cross (indicate parent varieties)[ ]					
		(ii)	partially known cross (indicate known parent varieties)					
		(iii)	unknown cross[ ]					
	(b) Mutation (indicate parent variety)		ation (indicate parent variety)[ ]					
	(c)	(c) Discovery and development (indicate where and when and how developed):						
	(d)	Oth	er (please provide details)[ ]					
4.2	Matk		- propagation					
4.2		(a)	propagation  Cuttings[ ]					
	(	(b)	In vitro propagation					
	(	(c)	Seed[ ]					
	(	(d)	Other (please specify):[ ]					

4.3	Virus sta	/irus status					
	(a)		all known viruses as follows uses)	[ ]			
	(b)		he plant material is virus tested indicate against which viruses)				
	(c)	The virus status is unkno	own	[ ]			
4.4	Geograp and devel		ty: the region and the country in which the variety was	bred or discovered			
5.		Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in the CPVO Protocol; please mark the state of expression which best corresponds).					
			or volved, please mark the state of expression w	mich best			
	correspon		Example varieties	Note			
5.1 (20)	correspon	nds).	· ·				
	correspon	aracteristics	·				
	Cha Fruit:	aracteristics	Example varieties	Note			
	Cha Fruit:	aracteristics	Example varieties  Wilking (HMA)	Note			
	Cha Fruit: short mediu long	aracteristics	Example varieties  Wilking (HMA)  Clemenules (CLE)	Note  3 [ ] 5 [ ]			
5.2	Cha Fruit: short mediu long	eracteristics length	Example varieties  Wilking (HMA)  Clemenules (CLE)	Note  3 [ ] 5 [ ]			
5.2	Cha Fruit: short mediu long Fruit:	aracteristics length m	Example varieties  Wilking (HMA)  Clemenules (CLE)  Minneola (TNL)	Note  3 [ ]  5 [ ]  7 [ ]			
5.2	Cha Fruit: short mediu long Fruit: small	aracteristics length m	Example varieties  Wilking (HMA)  Clemenules (CLE)  Minneola (TNL)  Clementina Fina (CLE)	Note  3 [ ]  5 [ ]  7 [ ]			
5.2	Chair Fruit: short mediu long Fruit: small mediu large	aracteristics length m	Example varieties  Wilking (HMA) Clemenules (CLE) Minneola (TNL)  Clementina Fina (CLE) Clemenules (CLE)	Note  3 [ ] 5 [ ] 7 [ ]  3 [ ] 5 [ ]			
5.2 (21)	Chair Fruit: short mediu long Fruit: small mediu large	diameter  presence of neck	Example varieties  Wilking (HMA) Clemenules (CLE) Minneola (TNL)  Clementina Fina (CLE) Clemenules (CLE)	Note  3 [ ] 5 [ ] 7 [ ]  3 [ ] 5 [ ]			

	Characteristics	Example varieties	Note
5.4 (39)	Fruit surface: predomin	nant colour(s)	
	green		1[]
	yellow green		2[]
	light yellow		3[]
	medium yellow	Mapo (TNL)	4[]
	yellow orange		5[]
	medium orange	Clemenules (CLE)	6[]
	dark orange		7[]
	orange red	Nova (HMA)	8[]
	red		9[]
5.5 (53)	Fruit: main colour of flo	esh	
(30)	whitish		1[]
	light green		2[]
	light yellow		3[]
	medium yellow	Mapo (HMA)	4[]
	light orange		5[]
	medium orange	Clemenules (CLE)	6[]
	dark orange		7[]
	red		8[]
	purple		9[]
5.6 (76)	Time of maturity of fru	it for consumption	
	early	Okitsu (SAT)	3[]
	medium	Clemenules (CLE)	5[]
	late	Murcott (TNR)	7[]
5.7 (77)	Parthenocarpy		
- •	absent	Wilking (HMA)	1[]
	present	Clemenules (CLE)	9[]

	Characterist	tics		Example varieties	Note
5.8 (78)	Self-incompa	tibility			
	absent		Común (MMN)		1[]
	present		Clemenules (CLE	≣)	9[]
6.	Similar varieties	and differences	from these varie	eties:	
	Denomination of similar variety		ic in which the ty is different <sup>1)</sup>	State of expression of similar variety	State of expression of candidate variety
1) I	n the case of identica	al states of expre	ssions of both vari	eties, please indicate the s	ize of the difference
7.	Additional inform	nation which ma	y help to disting	uish the variety	
A rep	resentative colour ph	otograph of the v	variety should acco	ompany the Technical Ques	stionnaire.
7.1	Resistance to pe	sts and disease	es		
7.2	Special condition	ns for the exam	ination of the va	ariety	
	[ ] YES,	please specify			
	[ ] NO				

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

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

Date Signature Name

[End of document]