TG/152/4(proj.3)
ORIGINAL: English
DATE: 2007-11-02

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

CHAMOMILE

UPOV Code: MATRI_REC

Matricaria recutita L.

*

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by an expert from Germany

to be considered by the Enlarged Editorial Committee at its meeting to be held in Geneva, Switzerland, on January 8, 2008

Alternative Names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Matricaria recutita L., *Chamomilla recutita* (L.) Rauschert	Chamomile	Camomille	Kamille	Manzanilla

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

* 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 Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Matricaria recutita* L. (*Chamomilla recutita* (L.) Rauschert).

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

5 g.

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should be stated by the applicant.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 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.

3. Method of Examination

3.1 Number of Growing Cycles

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

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 “Examining Distinctness”.

3.3 Conditions for Conducting the Examination

3.3.1 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.

3.3.2 The recommended method of observing the characteristics is indicated by the following key in the second column of the Table 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

3.4 Test Design

3.4.1 Each test should be designed to result in a total of at least 200 plants, which should be divided between two or more replicates.

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

3.5 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, all observations on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observations made on all plants in the test.

3.6 Additional Tests

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

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 Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.2 Uniformity

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 Cross-pollinated varieties

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

4.3 Stability

4.3.1 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 tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous 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)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

6. Introduction to the Table of Characteristics

6.1 Categories of Characteristics

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 States of Expression and Corresponding Notes

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 Types of Expression

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 Example Varieties

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

6.5 Legend

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3
QN: Quantitative characteristic – see Chapter 6.3
PQ: Pseudo-qualitative characteristic – see Chapter 6.3

MG, MS, VG, VS: See Chapter 3.3.2
C: special test

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

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

		English	français	Deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. (*) (+)	**MG C**	**Ploidy**	**Ploïdie**	**Ploidie**	**Ploidía**		
QL		diploid	diploïde	diploid	diploide	Camoflora	2
		tetraploid	tetraploïde	tetraploid	tetraploide	Manzana	4
2. (*) (+)	**VG**	**Plant: attitude of lower side shoots**	**Plante : port des pousses de la face inférieure**	**Pflanze: Haltung der unteren Seitentriebe**	**Planta: porte de los brotes laterales inferiores**		
QN	**(a)**	erect	dressé	aufrecht	erecto		1
		semi-erect	demi-dressé	halb aufrecht	semierecto	Mabamille	3
		horizontal	horizontal	waagerecht	horizontal		5
3. (*)	**MS**	**Plant: height**	**Plante: hauteur**	**Pflanze: Höhe**	**Planta: altura**		
QN	**(b)**	short	basse	niedrig	baja	Manzana	3
		medium	moyenne	mittel	media	Mabamille, Novbona	5
		tall	haute	hoch	alta	Lasyr	7
4.	**VG**	**Plant: density of foliage**	**Plante: densité du feuillage**	**Pflanze: Dichte des Laubes**	**Planta: densidad del follaje**		
QN	**(c)**	sparse	lâche	locker	laxa		3
		medium	moyenne	mittel	media	Bona	5
		dense	dense	dicht	densa	Bodegold, Lasyr	7
5.	**VG**	**Stem: anthocyanin coloration**	**Tige: pigmentation anthocyanique**	**Stängel: Anthocyanfärbung**	**Tallo: pigmentación antociánica**		
QN	**(a)**	weak	faible	gering	débil	Mabamille **(add.)**	3
		medium	moyenne	mittel	media	Bona, Novbona	5
		strong	forte	stark	fuerte	**no ex. variety for**	7

		English	français	Deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
6. (*)	**VG**	**Leaf: intensity of green color**	**Feuille: intensité de la couleur verte**	**Blatt: Intensität der Grünfärbung**	**Hoja: intensidad del color verde**		
QN	**(a)**	light	faible	hell	claro		1
		medium	moyenne	mittel	medio	Robumille	2
		dark	forte	dunkel	oscuro	Camoflora	3
7.	**VG**	**Leaf: division**	**Feuille: division**	**Blatt: Fiederung**	**Hoja: división**		
(+)							
QN	**(c)**	fine	fine	fein	fina		3
		medium	moyenne	mittel	mediana	Robumille	5
		coarse	grossière	grob	grosera		7
8. (*) (+)	**MS**	**Flower head: diameter**	**Capitule: diamètre**	**Blütenkopf: Durchmesser**	**Capítulo: diámetro**		
QN	**(a)**	small	petit	klein	pequeño	Bona	3
		medium	moyen	mittel	medio	Bodegold, Camoflora	5
		large	grand	groß	grande	Lasyr, Margaritar	7
9. (*)	**MS**	**Flower head: diameter of disc**	**Capitule: diamètre du disque**	**Blütenkopf: Durchmesser der Scheibe**	**Capítulo: diámetro del disco**		
QN	**(a)**	small	petit	klein	pequeño	Bodegold, Bona	3
		medium	moyen	mittel	medio	Robumille	5
		large	grand	groß	grande	Lasyr, Margaritar	7
10. (*) (+)	**MS**	**Time of beginning of flowering**	**Époque de début de floraison**	**Zeitpunkt des Blühbeginns**	**Época de comienzo de la floración**		
QN		early	précoce	früh	temprana	Camoflora	3
		medium	moyenne	mittel	media	Manzana	5
		late	tardive	spät	tardía	Zloty Lan	7

		English	français	Deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
11. (+)	**MS**	**Time of full flowering**	**Époque de pleine floraison**	**Zeitpunkt der Vollblüte**	**Época de comienzo**		
QN		early	précoce	früh	temprana	Bona	3
		medium	moyenne	mittel	media	Manzana	5
		late	tardive	spät	tardía	Bodegold	7
12. (+)	**MG**	**Flower head: amount of total essential oils**	**Capitule: teneur en huile essentielle**	**Blütenkopf: Gehalt an ätherischem Öl**	**Capítulo: cantidad total de aceites esenciales**		
QN	**(b)**	low	faible	niedrig	baja		3
		medium	moyenne	mittel	media	Robumille	5
		high	élevée	hoch	alta	Soroksári 40	7
13. (+)	**MG**	**Essential oil: amount of chamazulene**	**Huile essentielle: teneur en chamazulène**	**Ätherisches Öl: Gehalt an Chamazulen**	**Aceite esencial: cantidad de camazuleno**		
QN	**(b)**	low	faible	niedrig	baja		3
		medium	moyenne	mittel	media	Novbona	5
		high	élevée	hoch	alta	Mabamille	7
14. (+)	**MG**	**Essential oil: amount of (-)α-bisabolol**	**Huile essentielle: teneur en (-)α-bisabolol**	**Ätherisches Öl: Gehalt an (-)α-Bisabolol**	**Aceite esencial: cantidad de (-)α-bisabolol**		
QN	**(b)**	very low	très faible	sehr niedrig	muy baja	Bodegold, Camoflor, Promyk	1
		low	faible	niedrig	baja		3
		medium	moyenne	mittel	media		5
		high	élevée	hoch	alta	Manzana	7
		very high	très élévée	sehr hoch	muy alta	Mabamille, Novbona	9

8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

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

(a) The observations should be made at the time of beginning of flowering (See Ad. 10)
(b) The observations should be made at the time of full flowering
(c) The observations should be made at the flower bud stage

8.2 Explanations for individual characteristics

Ad. 1: Ploidy

The ploidy status of the plant can be determined by different methods as determination of the:

- number of chromosomes of the root meristem;
- number and length of stoma on the lower side of the leaf (tetraploid varieties have fewer stoma/mm² and longer stoma);
- number of chloroplasts in the guard cells on the lower side of the leaf (the guard cells of tetraploid varieties contain more chloroplasts than those of diploid varieties).

Another efficient method to determine the ploidy status is flow cytometry.

Ad. 2: Plant: attitude of lower side shoots

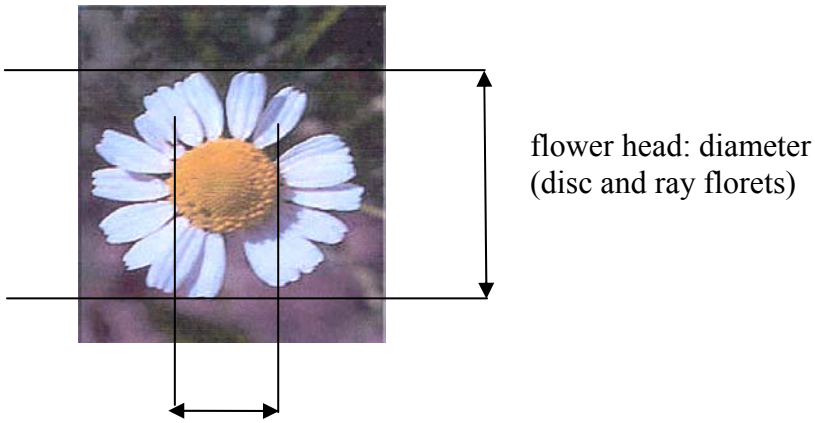
1 erect | 3 semi-erect | 5 horizontal

Ad. 7: Leaf: division

3	5	7
fine	medium	coarse

Ad. 8: Flower head: diameter
Ad. 9: Flower head: diameter of disc

The observation should be made at the beginning of flowering when the ray florets are horizontal.



Ad. 10: Time of beginning of flowering

The time of beginning of flowering of a given variety should be regarded as being reached if 20 % of the individual plants have ray florets developed in 5 flower heads of the plant.

Ad. 11: Time of full flowering

The evaluation should be done on individual plants. The individual plant should be regarded as having reached the stage of full flowering when 40 to 70 % of the disc flowers have opened in 50 % of the flower heads. The full flowering of a given variety has been reached when 80 % of the individual plants have reached the stage of full flowering.

Ad. 12: Flower head: amount of total essential oils

The amount of total essential oils is determined by vapor distillation using 30 g of dried flowers, a 1000 ml round-bottomed flask, 300 ml of *water R* as distillation liquid and 0.50 ml of *Xylol R* as receiver. Flowers should be prepared for drying not later than 2 hours after harvest to avoid decomposition of the active ingredients. The flowers can be air-dried in a drying loft or dried by technical means in a desiccator. The drying temperature should not exceed 45 °C, the residual moisture should be 7 % - 10 %. Distillation is carried out for 4 hours at a speed of 3 to 4 ml per minute. Towards the end of distillation the inflow of water to the cooling system is stopped, but the distillation is continued until the blue, steam-volatile components have reached the lower end of the cooling system. Immediately the cooling system is started again to prevent a warming of the separating flask. After a further 10 minutes, the distillation is terminated.

Ad. 13: Essential oil: amount of chamazulene
Ad. 14: Essential oil: amount of (-)α-bisabolol

The determination of chamazulene and (-)α-bisabolol is obtained by gas chromatography.

Definition

Blue essential oil obtained by steam distillation from the fresh or dried flower heads or flowering tops of *Matricaria recutita* L. (*Chamomilla recutita* L. Rauschert). There are 2 types of matricaria oil which are characterized as rich in bisabolol oxides, or rich in (-)α-bisabolol.

Characters

Appearance: clear, intensely blue, viscous liquid. It has an intense characteristic odour.

Tests

Chromatographic profile. Gas chromatography (2.2.28): use the normalization procedure.

Test solution: Dissolve 20 µl of the oil to be examined in *cyclohexane R* and dilute to 5.0 ml with the same solvent.

Reference solution: Dissolve 20 µl of *(-)α-bisabolol R*, 5 mg of *chamazulene R* and 6 mg of *guaiazulene R* in *cyclohexane R* and dilute to 5.0 ml with the same solvent.

Column:

- *material:* fused silica,

- *size:* l = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used), Ø = 0.25-0.53 mm, when using a column longer than 30 m, an adjustment of the temperature programme may be necessary,
- *stationary phase: macrogol 20 000 R.*

Carrier gas: helium for chromatography R.

Flow rate: 1-2 ml/min.

Split ratio: 1:100

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 40	70 → 230
	40 - 50	230
Injection port		250
Detector		250

Detection: flame ionisation

Injection: 1.0 µl

Elution order: order indicated in the composition of the reference solution. Record the retention times of these substances.

Relative retention with reference to chamazulene (retention time = about 34.4 min): β-farnesene = about 0.5; bisabolol oxide B = about 0.8: bisabolone = about 0.87; (-)α-bisabolol = about 0.9; bisabolol oxide A = about 1.02.

System suitability: reference solution:

- *resolution:* minimum 1.5 between the peaks due to chamazulene and guaiazulene.

Using the retention times determined from the chromatogram obtained with the reference solution, locate (-)α-bisabolol and chamazulene in the chromatogram obtained with the test solution; locate bisabolol oxides (bisabolol oxide B, bisabolone and bisabolol oxide A) using Figures 1836.-1 and 1836.-2 (disregard the peak due to cyclohexane). The chromatogram obtained with the test solution does not show a peak with the retention time of guaiazulene.

Determine the percentage content of the components. The limits are within the following ranges.

	Matricaria oil rich in bisabolol oxides (per cent)	Matricaria oil rich in (-)α-bisabolol (per cent)
Bisabolol oxides	29 - 81	
(-)α-bisabolol		10 - 65
Chamazulene	≥ 1.0	≥ 1.0
Total of bisabolol oxides and (-)α-bisabolol		≥ 20

Storage

In a well-filled, airtight container, protected from light at a temperature not exceeding 25 °C.

9. Literature

Carle, R., 1993: Bestimmung des Ploidiegrades von Kamillensorten durch cytomorphologische Methoden und mittels Durchfluß-Cytophotometrie. Vortr. Pflanzenzüchtung 26, pp. 42-48.

European Pharmacopoeia, 5th edition, Supplement 5.1, Published in accordance with the Convention on the Elaboration of a European Pharmacopoeia (European Treaty Series No. 50), European Directorate for the Quality of Medicines.

Schilcher, H., 1987: Die Kamille. Handbuch für Apotheker, Ärzte und andere Naturwissenschaftler, Wissenschaftliche Verlagsgesellschaft mbH Stuttgart.

10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire

1.1 Botanical name	*Matricaria recutita* L. (*Chamomilla recutita* (L.) Rauschert)
1.2 Common name	Chamomile

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	
Breeder (if different from applicant)	

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

[#]4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)

(b) partially known cross [ ]
(please state known parent variety(ies))

(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery and development [ ]
(please state where and when discovered and how developed)

4.1.4 Other [ ]
(please provide details)

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

(a) Cross-pollination
(i) population [ ]
(ii) synthetic variety [ ]

(b) Hybrid [ ]

(c) Other [ ]
(please provide details)

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

	Characteristics	Example Varieties	Note
5.1 (1)	**Ploidy**		
	diploid	Camoflora	2[ ]
	tetraploid	Manzana	4[ ]
5.2 (3)	**Plant: height**		
	short	Manzana	3[ ]
	medium	Mabamille, Novbona	5[ ]
	tall	Lasyr	7[ ]
5.3 (8)	**Flower head: diameter**		
	small	Bona	3[ ]
	medium	Bodegold, Camoflora	5[ ]
	large	Lasyr, Margaritar	7[ ]
5.4 (10)	**Time of beginning of flowering**		
	early	Camoflora	3[ ]
	medium	Manzana	5[ ]
	late	Zloty Lan	7[ ]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example	*Flower head: diameter*	*small*	*medium to large*

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

[#]7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [ ] No [ ]

(If yes, please provide details)

7.3 Other information

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

If the answer to (b) is yes, please attach a copy of the authorization.

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

9. Information on plant material to be examined or submitted for examination.

9.1 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.

9.2 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, pesticide) Yes [ ] No [ ]

(c) Tissue culture Yes [ ] No [ ]

(d) Other factors Yes [ ] No [ ]

Please provide details for where you have indicated "yes".

……

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature Date

[End of document]