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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS GENEVA



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 Technical Working Party for Vegetables at its forty-first session, to be held in Nairobi, Kenya, from June 11 to 15, 2007

Alternative Names:*

Botanical nameEnglishFrenchGermanSpanishMatricaria recutita L.,
Chamomilla recutita (L.) RauschertCamomileKamilleManzanilla

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:

Cross-pollinated varieties

4.2.2 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. <u>Introduction to the Table of Characteristics</u>

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
- ON: Ouantitative 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. <u>Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres</u>

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. (*) (+)	VS C	Ploidy	Ploïdie	Ploidie			
QL		diploid	diploïde	diploid		Camoflora	2
		tetraploid	tetraploïde	tetraploid		Manzana	4
2. (*) (+)	VG	Plant: attitude of lower side shoots	Plante:	Pflanze: Haltung der unteren Seitentriebe	Planta:		
QN	(a)	erect		aufrecht			1
		semi-erect		halb aufrecht		Mabamille	3
		prostrate		waagerecht			5
3. (*)	MS	Plant: height	Plante: hauteur	Pflanze: Höhe	Planta: altura		
QN	(c)	short	basse	niedrig	baja	Manzana	3
		medium	moyenne	mittel	media	Novbona, Mabamille	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	(b)	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: Anthocyanfär-bung	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: green color	Feuille: couleur verte	Blatt: Grünfärbung	Hoja: color verde		
PQ	(a)	light green		hellgrün			1
		medium green		mittelgrün		Robumille	2
		dark green		mittelgrün		Camoflora	3
		grey green		graugrün			4
7.	VG	Leaf: division	Feuille: division	Blatt: Fiederung	Hoja: división		
(+) (add.)							
QN	(b)	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			
QN	(c)	small	petit	klein	pequeño	Bona	3
		medium	moyen	mittel	medio	Bodegold, Camoflora	5
		large	grand	groß	grande	Lasyr, Margaritar	7
				of the flower head me t the beginning of the		ndrical and ray flower	s. It has
9. (+)	VG	Ray floret: shape of apex	Fleur ligulée: forme du sommet	Zungenblüte: Form der Spitze			
QL	(c)	rounded	arrondi	rundlich			1
		crenate		gekerbt			2
DE: p	ropos	al to delete char. 9, no	differences between t	the varieties, HU agree	es		
10. (*)	MS	Flower head: diameter of disc	Capitule: diamètre du disque	Blütenkopf: Durchmesser der Scheibe			
QN	(c)	small	petit	klein	pequeño	Bodegold, Bona	3
	()						
	()	medium	moyen	mittel	medio	Robumille	5

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		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. +)	VG	Flower head: curvature of disc	Capitule: courbure du disque	Blütenkopf: Wölbung der Scheibe			
)N	(c)	weak		gering			3
		medium		mittel			5
		strong		stark			7
E: p	roposa	al to delete char. 11, n	o differences between	the varieties. Its mor	e a question of the dev	elopment stage, HU a	grees
E: p. 12. (*) (+)	mS		o differences between É Époque de début de floraison		e a question of the dev Época de comienzo de la floración	elopment stage, HU a	grees
2. (*) (+)		Time of beginning of	Époque de début de	Zeitpunkt des	Época de comienzo	elopment stage, HU as	grees 3
12. (*)		Time of beginning of flowering	Époque de début de floraison	Zeitpunkt des Blühbeginns	Época de comienzo de la floración		
2. *) +)		Time of beginning of flowering	É Époque de début de floraison précoce	Zeitpunkt des Blühbeginns	Época de comienzo de la floración temprana	Camoflora	3
2. (*) (*) (*) (N)		Time of beginning of flowering early medium	Époque de début de floraison précoce moyenne	Zeitpunkt des Blühbeginns früh mittel	Época de comienzo de la floración temprana media	Camoflora Manzana	3 5
2. (*) (+)	MS	Time of beginning of flowering early medium late Time of full	Époque de début de floraison précoce moyenne tardive Époque de pleine	Zeitpunkt des Blühbeginns früh mittel spät Zeitpunkt der	Época de comienzo de la floración temprana media tardía	Camoflora Manzana	3 5

DE: proposal to delete char. 13, HU agrees

tardive

late

14.	MG	Flower head: amount of total	Capitule: teneur en huile essentielle	Blütenkopf: Gehalt an ätherischem Öl		
(+)		essential oils				
QN	(c)	low	faible	niedrig		3
		medium	moyenne	mittel	Robumille	5
		high	élevée	hoch	Soroksári 40	7
15.	MG	Essential oil:	Huile essentielle: teneur en	Ätherisches Öl: Gehalt an		
(+)		chamazulene	chamazulène	Chamazulen		
QN	(c)	low	faible	niedrig		3
		medium	moyenne	mittel	Novbona	5

spät

tardía

Bodegold

7

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		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
16. (+)	MG	Essential oil: amount of (-)α-bisabolol	Huile essentielle: teneur en (-)α-bisabolol	Ätherisches Öl: Gehalt an (-)α-Bisabolol			
QN	(c)	very low	très faible	sehr niedrig		Bodegold, Camoflor (add.), Promyk	1
		low	faible	niedrig			3
		medium	moyenne	mittel			5
		high	élevée	hoch		Manzana (add.)	7
		very high	très élévée	sehr hoch		Mabamille, Novbona (add.)	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. 12)
- (b) The observations should be made at the flower bud stage.
- (c) The observations should be made at the 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 cylindrical 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.

8.2 Explanations for individual characteristics

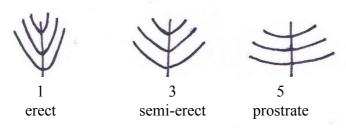
Ad. 1: Ploidy

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

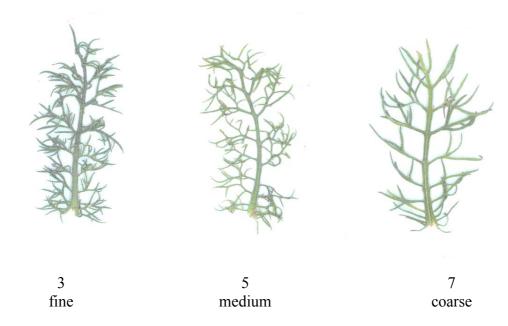
- of chromosomes of the root meristem
- and length of stoma on the lower side of the leaf (tetraploid varieties have a fewer number of stoma/mm² and a bigger length of stoma)
- of chloroplasts in the guard cells on the lower side of the leaf (the guard cells of tetraploid varieties contain more chloroplasts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

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

Ad. 2: Plant: attitude of lower side shoots



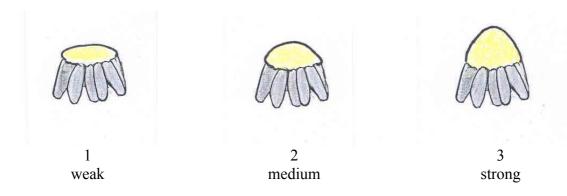
Ad. 7: Leaf: division



Ad. 9: Ray floret: shape of apex – to be deleted



Ad. 11: Flower head: curvature of disc – to be deleted



Ad. 12: Time of beginning of flowering

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

Ad. 13: to be deleted and added to 8.1

Ad. 14: Flower head: content of essential oil

The content of essential oil 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. Distillation is carried out for 4 h at a speed of 3 to 4 ml per minute. Towards end of distillation the inflow of water to the cooling system has to be stopped, but the distillation to be continued until the blue, steam-volatile components have reached the lower end of the cooling system. Immediately the cooling system has to be started again to prevent a warming of the separating flask. After further 10 minutes the distillation is to be terminated.

Ad. 15: Essential oil: amount of chamazulene Ad. 16: Essential oil: amount of (-)α-bisabolol

The determination of chamazulene and $(-)\alpha$ -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 characterised as rich in bisabolol oxides, or rich in $(-)\alpha$ -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 μ l 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: 1 = 30 m (a film thickness of 1 μ m may be used) to 60 m (a film thickness of 0.2 μ m may be used), $\emptyset = 0.25$ -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 $(-)\alpha$ -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.

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	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.

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9. <u>Literature</u>

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

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10. <u>Technical Questionnaire</u>

TECHNICAL QUESTION	NAIRE	Page {x} of {y}	Reference Number:
			Application date: (not to be filled in by the applicant)
to be completed		INICAL QUESTIONN tion with an applicatio	NAIRE on for plant breeders' rights
1. Subject of the Techni	cal Quest	ionnaire	
1.1 Botanical name		ttricaria recutita L. hamomilla recutita (L.) Rauschert)
1.2 Common name	CH	IAMOMILE	
2. Applicant			
Name			
Address			
Telephone No.			
Fax No.			
E-mail address			
Breeder (if different f	rom appli	cant)	
3. Proposed denomination	on and bro	eeder's reference	
Proposed denomination (if available)	on		
Breeder's reference			

TEC	CHNIC	CAL QI	UESTIONNAIRE	Page {x} of {y}	Reference Number:		
[#] 4.	*4. Information on the breeding scheme and propagation of the variety						
	4.1	Breedi	ng scheme				
		Variet	y resulting from:				
		4.1.1	Crossing				
			(a) controlled of (please state	eross e parent varieties)	[]		
			(b) partially kn (please state	own cross e known parent variety([]		
			(c) unknown c	ross	[]		
		4.1.2	Mutation (please state pare	nt variety)	[]		
		4.1.3	Discovery and de (please state whe and how develop	re and when discovered	[]		
		4.1.4	Other (please provide d	etails)	[]"		

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

TECHNICAL QUE	STIONNAIRE	Page {x} of {y}	Reference Number:	
4.2 Method of pro	pagating the varie	ety		
4.2.1 Se	ed-propagated var	rieties		
(a)	Cross-pollinat (i) population (ii) synthetic	n	[]	
(b)	Hybrid		[]	
(d)	Other (please provid	le details)	[]	

TECH	HNICAL QUESTIONNAIRE	Page {x} of {y}	Reference	Number:	
	Characteristics of the variety t sponding characteristic in Te sponds).				
	Characteristics			Example Varieties	Note
5.1 (1)	Ploidy				
	diploid			Camoflora	2[]
	tetraploid			Manzana	4[]
5.2 (3)	Plant: height				
	short			Manzana	3[]
	medium			Novbona, Mabamille	5[]
	tall			Lasyr	7[]
5.3 (8)	Flower head: diameter				
	small			Bona	3[]
	medium			Bodegold, Camoflora	5[]
	large			Lasyr, Margaritar	7[]
5.4 (12)	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.	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.

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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							
	Signa	ture		Date			

[End of document]