E

TG/152/4
ORIGINAL: English
DATE: 2008-04-09

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

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.]

TABLE OF CONTENTS PAGE

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 The assessment of uniformity for cross-pollinated varieties 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: density of foliage**	**Plante: densité du feuillage**	**Pflanze: Dichte des Laubes**	**Planta: densidad del follaje**		
QN	**(c)**	sparse	lâche	locker	escasa		3
		medium	moyenne	mittel	media	Bona	5
		dense	dense	dicht	densa	Bodegold, Lasyr	7
3. (*) (+)	**VG**	**Plant: attitude of lower side shoots**	**Plante : port des rameaux latéraux inférieurs**	**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
4. (*)	**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
5.	**VG**	**Stem: anthocyanin coloration**	**Tige: pigmentation anthocyanique**	**Stängel: Anthocyanfärbung**	**Tallo: pigmentación antociánica**		
QN	**(a)**	weak	faible	gering	débil	Mabamille	3
		medium	moyenne	mittel	media	Bona, Novbona	5
		strong	forte	stark	fuerte		7

		English	français	Deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
6. **(+)**	**VG**	**Leaf: division**	**Feuille: division**	**Blatt: Fiederung**	**Hoja: división**		
QN	**(c)**	coarse	grossière	grob	gruesa		3
		medium	moyenne	mittel	mediana	Robumille	5
		fine	fine	fein	fina		7
7. **(*)**	**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
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: content of (-)α-bisabolol in essential oil**	**Capitule: teneur en (-)α-bisabolol dans l'huile essentielle**	**Blütenkopf: Gehalt an (-)α-Bisabolol im ätherischen Öl**	**Capítulo: contenido de (-)α-bisabolol en el aceite esencial**		
QN	**(b)**	low	faible	niedrig	baja	Bodegold, Camoflora, Margaritar	1
		medium	moyenne	mittel	media	Promyk	2
		high	élevée	hoch	alta	Manzana, Novbona, Robumille	3

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 flower bud stage.
(b) The observations should be made at the time of beginning of flowering. (See Ad. 10)
(c) The observations should be made at the time 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;
- 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. 3: Plant: attitude of lower side shoots

1 erect | 3 semi-erect | 5 horizontal

Ad. 6: Leaf: division

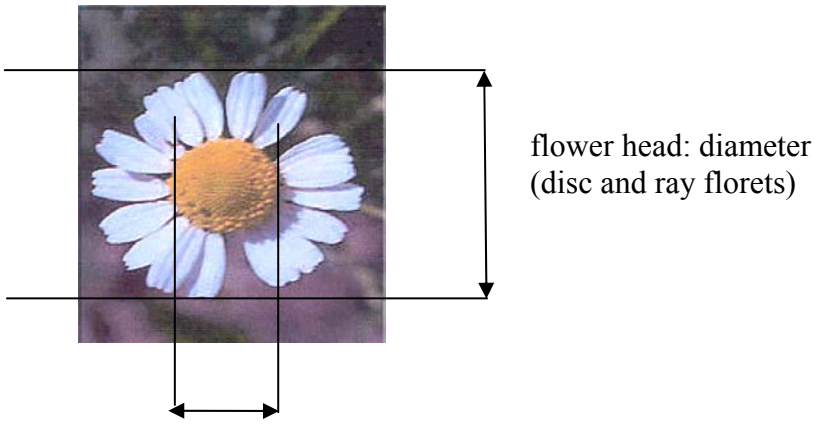
3
coarse

5
medium

7
fine

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: content of (-)α-bisabolol in essential oil

The essential oil should be extracted by vapor distillation from 30g of dried flower heads. The amount of (-)α-bisabolol in the essential oil should be analyzed by gas chromatography.

Flowers should be prepared for drying not later than 2 hours after harvest to avoid decomposition of the oil. The drying temperature should not exceed 45°C. The residual moisture should be 7%-11%. Distillation conditions: distillation liquid 300ml *water R* and 0.5ml *Xylol R* as receiver; 4 hours with 3-4ml per minute.

The clear, intensively blue, viscous essential oil is separated by gas chromatography. Appropriate gas chromatic methods are reviewed by Schilcher (1987).

The main components of the essential oil of chamomile are: (-)α-bisabolol, chamaculen, bisabolol oxide A, bisabolol oxide B and bisabolon oxide A. In relation to (-)α-bisabolol, there are 3 levels:

Note	Level	Content of (-)α-bisabolol in the essential oil	Example varieties
1	low	≤ 5%	Bodegold, Camoflora, Margaritar
2	medium	5%-30%	Promyk
3	high	>30%	Manzana, Novbona, Robumille

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)

4.2.2 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 (4)	**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]