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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

EUCALYPTUS

EUCAL

(Sub-genus *Symphyomyrtus)*
(Sections *Transversaria*, *Maidenaria*, *Exsertaria*)

*

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from Brazil

to be considered by the
Technical Working Party for Ornamental Plants and Forest Trees
at its forty-first session, to be held in Wageningen, Netherlands, from June 9 to 13, 2008

Alternative Names:*

Botanical Name	*English*	*French*	*German*	*Spanish*
	Eucalyptus			

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 vegetative propagated varieties of the species of the sections *Transversaria*, *Maidenaria* and *Exsertaria* of the sub-genus *Symphyomyrtus* of the genus *Eucalyptus*.

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 young plants, 3 to 6 months old.

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

10 plants, properly packaged to minimize damage.

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

3.1.1 The minimum duration of tests should normally be a single growing cycle.

3.1.2 The growing cycle is considered to be the period ranging from the beginning of active vegetative growth, continuing through active vegetative growth and concluding with fruiting.

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 plants must be planted in the ground as soon as the material is supplied by the breeder.

3.3.3 The observations on 44 and 68 month old plants should be made on plants of the clone selected during the breeding process (clonal tests).

3.3.4 The plants used in the test should be obtained from the same clones being utilized simultaneously in the 44 and 68 month old tests.

3.4 Test Design

3.4.1 The recommended spacing is 3 x 3 meters.

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

3.5.1 Unless otherwise indicated, all observations should be made on 5 plants or parts taken from each of 5 plants.

3.5.2 Observations on leaves should be made on leaves located on terminal shoots in vegetative growth.

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 For the assessment of uniformity, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 5 plants, no off-types are allowed.

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 plant 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) Leaf: petiole (characteristic 1)
(b) Leaf: anthocyanin (characteristic 10)
(c) Leaf: waxiness (characteristic 18)
(d) Primary branch: type of insertion in main stem (characteristic 19)

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

(a)- (d) 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. **(*)** **(+)**		**Leaf: petiole**					
QL	**(a)**	absent					1
		present					9
2.		**Leaf blade: length**					
QN	**(a)**	short					3
		medium					5
		long					7
3. **(+)**		**Leaf blade: width**					
QN	**(a)**	narrow					3
		medium					5
		broad					7
4.		**Leaf blade: ratio width/ length**					
QN	**(a)**	small					3
		medium					5
		large					7
5.		**Leaf blade: position of broadest part**					
QN	**(a)**	towards top					3
		at middle					5
		towards base					7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
6. **(+)**		**Leaf blade: shape of base**					
PQ	**(a)**	cuneate					1
		attenuate					2
		obtuse					3
		cordate					4
		auriculate					5
		hastate					6
		sagittate					7
		oblique					8
		peltate					9
		connate					10
		amplexicaul					11
		decurrent					12
7. **(+)**		**Leaf blade: shape of apex**					
PQ	**(a)**	subulate					1
		aristate					2
		acuminate					3
		mucronate					4
		apiculate					5
		acute					6
		obtuse					7
		emarginate					8

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
8.		**Leaf blade: tip**					
QL	**(a)**	absent					1
		present					9
9.		**Leaf: waxiness on upper side**					
QN	**(a)**	absent or weak					1
		medium					2
		strong					3
10. (*)		**Leaf: anthocyanin**					
QN	**(a)**	absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
11. (+)		**Leaf: attitude of blade**					
PQ	**(b)**	upwards					1
		outwards					2
		downwards					3
12.		**Leaf blade: length**					
(+)							
QN	**(b)**	short					3
		medium					5
		long					7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
13.		**Leaf blade: width**					
(+)							
QN	**(b)**	narrow					3
		medium					5
		broad					7
14.		**Leaf blade: ratio width/ length**					
QN	**(b)**	small					3
		medium					5
		large					7
15.		**Leaf blade: position of broadest part**					
QN	**(b)**	towards top					3
		at middle					5
		towards base					7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
16.		**Leaf blade: shape of base**					
(+)							
PQ	**(b)**	cuneate					1
		attenuate					2
		obtuse					3
		cordate					4
		auriculate					5
		hastate					6
		sagittate					7
		oblique					8
		peltate					9
		connate					10
		amplexicaul					11
		decurrent					12
17.		**Leaf blade: shape of apex**					
(+)							
PQ	**(b)**	subulate					1
		aristate					2
		acuminate					3
		mucronate					4
		apiculate					5
		acute					6
		obtuse					7
		emarginate					8

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
18. (*)		**Leaf: waxiness**					
QN	**(b)**	absent or weak					1
		medium					2
		strong					3
19. (*) (+)		**Primary branch: type of insertion in main stem**					
PQ	**(b)**	inverted “V”					1
		spherical					2
20.		**Branch: attitude**					
PQ	**(a)**	erect					1
		semi-erect					2
		horizontal					3
21.		**Trunk: rythidome**					
(+)							
QL	**(c)**	absent					1
		present					9
22.		**Trunk: waxiness**					
QL	**(b)**	absent					1
		present					9
23.		**Only for varieties with rhytidome: Trunk: predominant color of rythidome**					
PQ	**(d)**	green					1
		brown					2
		gray					3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24.		**Trunk: predominant color**					
PQ	**(d)**	cream					1
		green					2
		bluish green					3
		brown					4
		gray					5
25.		**Leaf: attitude**					
(+)							
QN	**(c)**	upwards					1
		outwards					2
		downwards					3
26.		**Leaf blade: length**					
(+)							
QN	**(c)**	short					3
		medium					5
		long					7
27.		**Leaf blade: width**					
(+)							
QN	**(c)**	narrow					3
		medium					5
		broad					7
28.		**Leaf blade: ratio width/ length**					
QN	**(c)**	small					3
		medium					5
		large					7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
29.		**Leaf blade: position of broadest part**					
QN	**(c)**	towards top					3
		at middle					5
		towards base					7
30.		**Leaf blade: shape of base**					
(+)							
PQ	**(c)**	cuneate					1
		attenuate					2
		obtuse					3
		cordate					4
		auriculate					5
		hastate					6
		sagittate					7
		oblique					8
		peltate					9
		connate					10
		amplexicaul					11
		decurrent					12

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
31. **(+)**		**Leaf blade: shape of apex**					
PQ	**(c)**	subulate					1
		aristale					2
		acuminate					3
		mucronate					4
		apiculate					5
		acute					6
		obtuse					7
		emarginate					8
32.		**Leaf: intensity of color of upper side in relation to lower side**					
PQ	**(c)**	same					1
		darker					2
33.		**Leaf: waxiness of upper side**					
QN	**(c)**	absent or weak					1
		medium					2
		strong					3
34. **(*)** **(+)**		**Primary branch: on lower third crow type of insertion in main stem**					
PQ	**(d)**	inverted “V”					1
		spherical					2

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
35. **(+)**	**Tree: age at first flowering**					
QN	young					1
	medium					2
	old					3
36.	**Flower arrangement**					
QL	single					1
	umbel					2
37.	**Only varieties with umbel flower arrangement: Umbel: number of buds**					
PQ	three					1
	seven					2
	nine					3
	eleven					4
	> eleven					5
38. **(+)**	**Umbel: peduncle**					
QL	absent					1
	present					9
39.	**Only varieties with umbel with peduncle: Peduncle: length**					
QN	short					3
	medium					5
	long					7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
40.	**Umbel: shape of peduncle in cross section**					
QL	rounded					1
	flattened					2
41.	**Flower bud: shape of opercule**					
(+)						
PQ	rostrate					1
	hemispherical					2
	hemispherical apiculate					3
	flattened with a prominent pointed tip					4
	horn-shaped					5
	elongated					6
	conical					7
42.	**Fruit: pedicel**					
(+)						
QL	absent					1
	present					9
43.	**Fruit: length of pedicel in relation to the length of calyx**					
QN	shorter					1
	equal					2
	longer					3

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
44.	**Fruit: size**					
(+)						
QN	small					3
	medium					5
	large					7
45.	**Fruit: shape**					
(+)						
PQ	conical					1
	pyriform					2
	cylindrical					3
	urceolate					4
	globose					5
	hemispherical					6
	campanulate					7
	ovoid					8
46.	**Fruit: texture of surface**					
(+)						
QL	smooth					1
	rough					2
47.	**Fruit: disc**					
(+)						
QL	descending					1
	same level					2
	ascending					3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
48.		**Fruit: valve position**					
(+)							
QL		sunken					1
		rim level					2
		exserted					3
49. **(+)**		**Only for varieties with rhytidome: Trunk: texture of basal rythidome**					
PQ	**(d)**	rough/compact					1
		rough/fibrous					2
50.		**Only for varieties with rhytidome: Trunk: height of rhytidome**					
QN	**(d)**	lower third					3
		medium third					5
		upper third					7
51.		**Wood: density**					
(+)							
QN	**(d)**	low					3
		medium					5
		high					7

8. Explanations on the Table of Characteristics

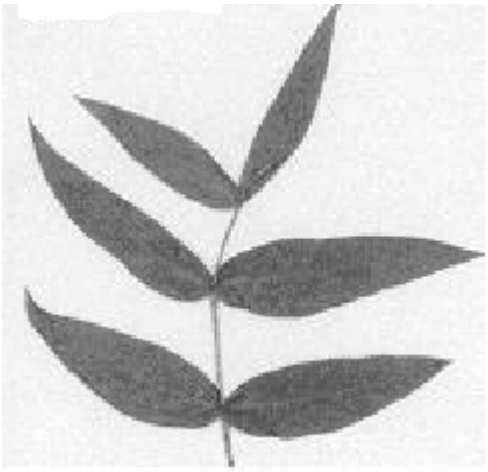
8.1 Explanations covering several characteristics

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

(a) All observations should be made on 11 to 12 months old
(b) All observations should be made on plants at 18 to 20 months old.
(c) All observations should be made on plants 44 months old.
(d) All observations should be made on plants 68 months old.

8.2 Explanations for individual characteristics

Ad. 1: Leaf: petiole



1
absent

9
present

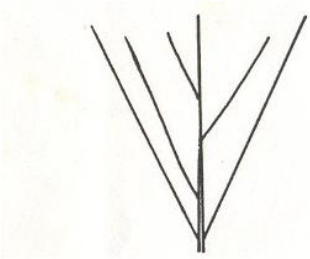
Ad. 3, Ad. 13, Ad. 27: Leaf blade: width

3
narrow

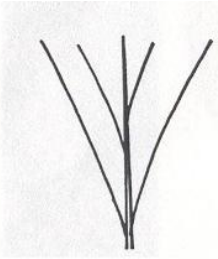
5
medium

7
broad

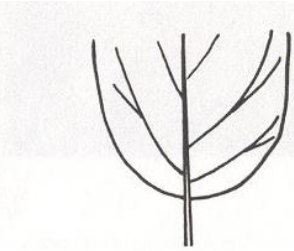
Ad. 6, 16, 30: Leaf: shape of base



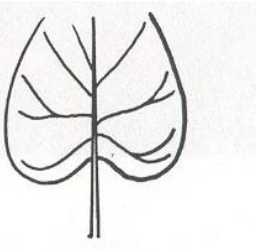
1
cuneate



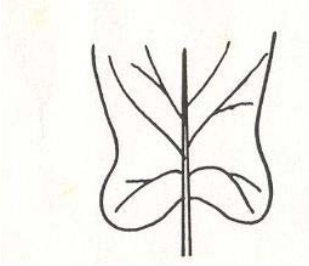
2
attenuate



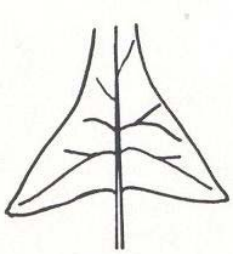
3
obtuse



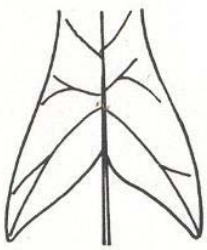
4
cordate



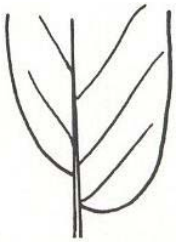
5
auriculate



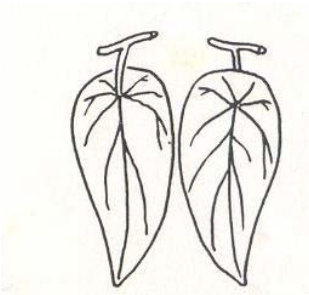
6
hastate



7
sagittate

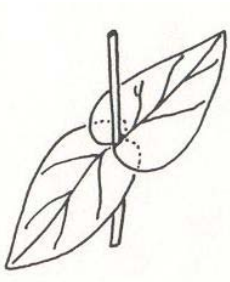


8
oblique



9
peltate

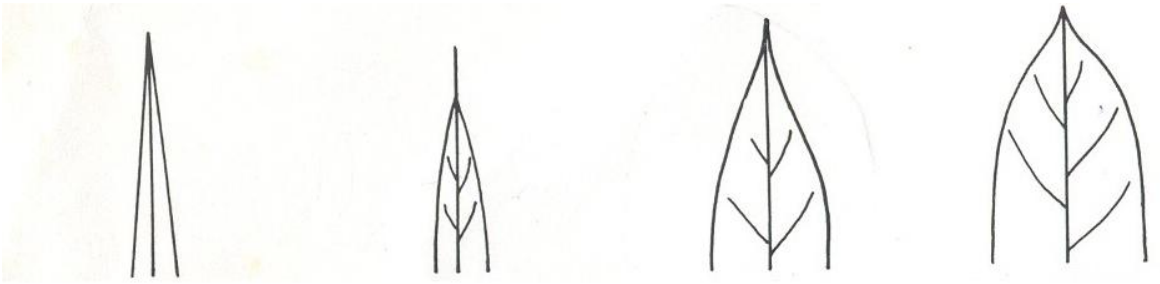
10
connate



11
amplexicaul

12
decurrent

Ad. 7, Ad. 17, Ad. 31: Leaf: shape of apex

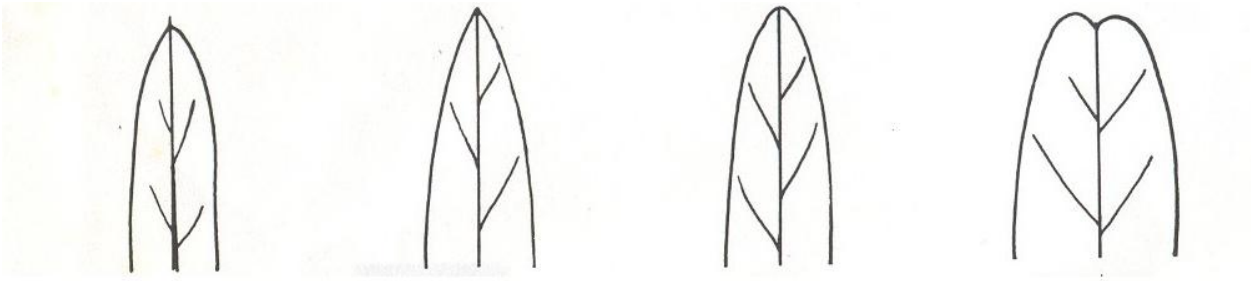


1
subulate

2
aristate

3
acuminate

4
mucronate



5
apiculate

6
acute

7
obtuse

8
emarginate

Ad. 11: Leaf: attitude of blade
Ad. 25: Leaf: attitude

This characteristic should be observed with the branch positioned vertically.

1
upwards

2
outwards

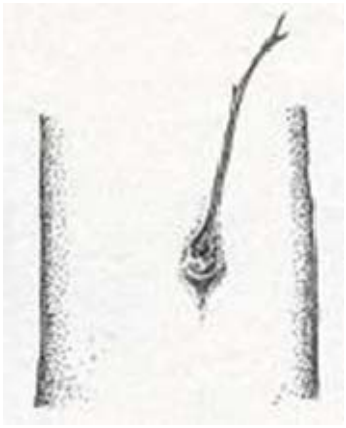
3
downwards

Ad. 12., Ad. 26: Leaf blade: length

The length should be evaluated on the biggest leaf of a branch located in the beginning of the upper third of the crown in 3 year old plants.

Ad. 19: Primary branch: type of insertion in main stem
Ad. 34: Primary branch: on lower third crow type of insertion in main stem

1
inverted "V"



2
spherical

Ad. 21: Trunk: rhytidome

As a tree grows in diameter, the bark tissues are stretched and eventually crack. A new phellogen is then originated in the phloem, and the tissues outside this new layer die and dry out, thus forming part of the outer rough bark of the tree. This bark is known as the rhytidome. In the strictest sense, the rhytidome is the true bark of the tree, as the inner soft tissue of the bark is actually the phloem.

Ad. 27: Leaf blade: width

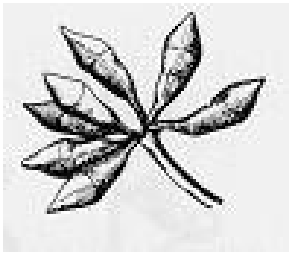
This evaluation should be performed on the same leaf selected for characteristic 26. The observation should be made in the widest part of the blade.

Ad. 35: Tree: age at first flowering

1: young: under two years
2: medium: between two and four years
3: old: more than four years

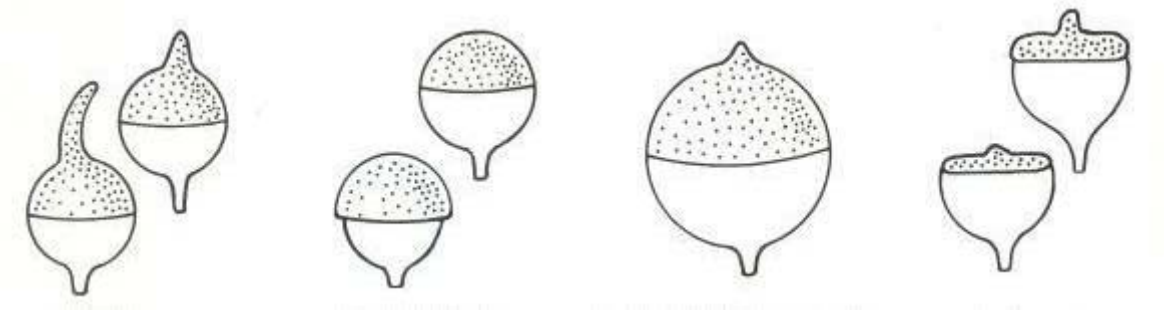
Ad. 38: Umbel: peduncle

[to be provided]

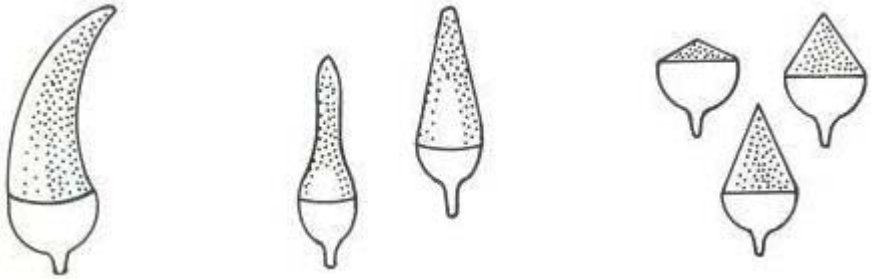


1	2
absent	present

Ad. 41: Flower bud: shape of opercule

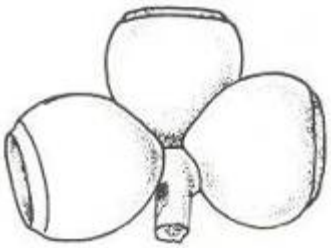


1	2	3	4
rostrate	hemispherical	hemispherical apiculate	flattened with a prominent pointed tip



5	6	7
horn-shaped	elongated	conical

Ad. 42: Fruit: pedicel



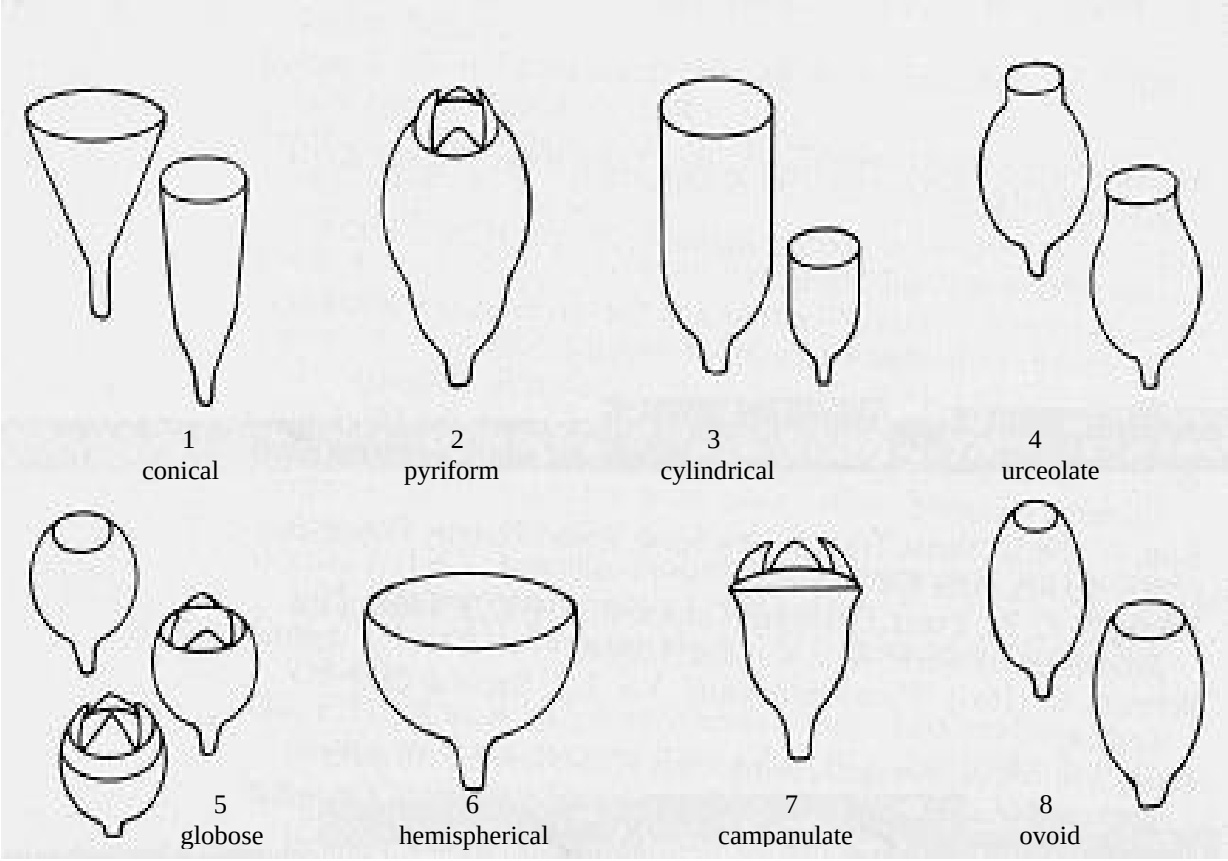
1
absent

9
present

Ad. 44: Fruit: size

The size of the fruit is observed on the basis of the largest diameter.

Ad. 45: Fruit: shape

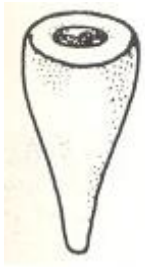


Ad. 46: Fruit: texture of surface

The texture of the fruit should be observed during current year fruitage.

Ad. 47: Fruit: disc

1
descending



2
same level

3
ascending

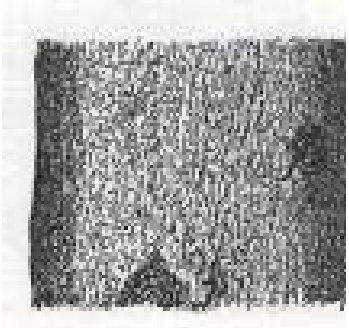
Ad. 48: Fruit: valve position

1
sunken

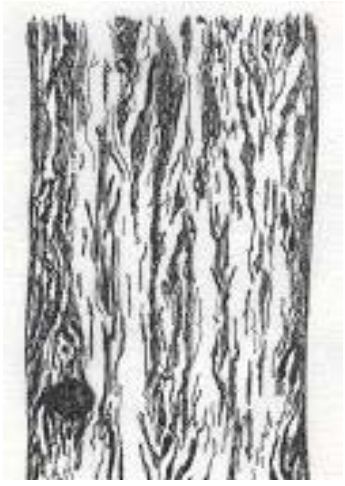
2
rim level

3
exserted

Ad. 49: Only for varieties with rhytidome: Trunk: texture of basal rythidome



1
rough/compact



2
rough/fibrous

Ad. 51: Wood: density

The density must be evaluated based on the wood volume at the highest level of humidity, through the hydrostatic balance methodology, according to TAPPI Norm #T258 om-94 (Technical Association of Pulp and Paper Industry).

9. Literature

Boland, D. J.; Brooker, M. I. H.; Chippendale, G. M.; Hall, N.; Hyland, B. P. M.; Johnston, R. D., Kleinig, D. A. & Turner, J. D., 1994: Forest trees of Autralia. 4ª ed. Melbourne: Nelson: CSIRO. 703 p.

Brooker, M.I.H. & Kleinig, D.A., 1999: Field guide to eucalypts: south – eastern Australia Vol. 1. 2ª ed. Bloomings Books. 353 p.

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Chippendale, G. M., 1968: Eucalyptus buds and fruits. Canberra: Forestry and Timber Bureau. 96 p.

FAO,1981: El eucalipto en la repoblación forestal. Roma, IT, 723 p.

Goes, E., 1985: Os Eucaliptos. Lisboa, 372 p.

Penfold, A.R. & Willis, J.L., 1961: The Eucalypts. New York, 551 p.

Drawings by: Anna Júlia Passold, Israel Gomes Vieira and Joel F. Penteado Jr.

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 Genus	*Eucalyptus*
1.2 Sub-genus	*Symphyomyrtus*
1.3 Section	Transversaria – Exsertaria - Maidenaria
1.4 Species (please complete)	

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 Vegetative propagation

(a) cuttings [ ]
(b) *in vitro* propagation [ ]
(c) other (state method) [ ]

4.2.2 Seed [ ]

4.2.3 Other [ ]

[#] 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)	**Leaf: petiole**		
	absent		1 [ ]
	present		9 [ ]
5.2 (19)	**Primary branch: type of insertion in main stem**		
	inverted “V”		1 [ ]
	spherical		2 [ ]
5.3 (37)	**Only varieties with umbel flower arrangement: Umbel: number of buds**		
	three		1 [ ]
	seven		2 [ ]
	nine		3 [ ]
	eleven		4 [ ]
	> eleven		5 [ ]
5.4 (45)	**Fruit: shape**		
	conical		1 [ ]
	pyriform		2 [ ]
	cylindrical		3 [ ]
	urceolate		4 [ ]
	globose		5 [ ]
	hemispherical		6 [ ]
	campanulate		7 [ ]
	ovoid		8 [ ]

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

	Characteristics	Example Varieties	Note
5.5 (49)	**<u>Only for varieties with rhytidome</u>: Trunk: texture of basal rythidome**		
	rough/compact		1 [ ]
	rough/fibrous		2 [ ]
5.6 (?)	**Tree: texture of basal bark on lower part (5 years it is very defined)**		
	rough/compact		1 [ ]
	rough/fibrous		2 [ ]

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	*[insert example]*	*[insert example]*	*[insert example]*

Comments:

[#]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

[#] 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:

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.

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

[Annex follows]

ANNEX

Additional Useful Explanations

PART I

Introduction

The following Annex contains the characteristics of molecular descriptors to be used for the identification of clones and varieties of *Eucalyptus.* A molecular description has as primary objective, to determine the genetic profile of plants pertaining to the Eucalyptus genus through the analysis of multiple loci in the DNA. Twenty-five loci markers using microsatellite sequences are recommended, and are to be considered as complementary descriptors for the identification of clones, hybrids and varieties of Eucalyptus. The molecular characterization of these loci have been already published in the literature and are being widely used in several laboratories around the world, aiming primarily to the identification of individual trees of *Eucalyptus,* pertaining to almost all the commercially relevant species of sub-genus *Symphyomyrtus*, *Idiogenes* and *Monocalyptus.*

PART II

Characteristics of molecular descriptors

For determination of the genetic profile of a sample, twenty-five microsatellite loci are recommended, according to the table below, to allow a standardization of the genetic profiles generated. At least two molecular markers are listed for each of the eleven linkage groups, corresponding to the eleven chromosomes of *Eucalyptus, but* the analyst can utilize only as many loci as considered necessary for his/her specific situation, looking first for genetically independent markers (in other words, for different linkage groups). However, to allow comparisons among several testing laboratories, it is important for the user to utilize only recommended markers. The higher the number of loci used, the geater the power of discrimination, allowing for more certainty in the identification and comparison process. These loci were published and optimized for genetic identification purposes in *Eucalyptus* (*Brondani, R.P.V., Brondani, C., Tarchini, R., Grattapaglia, D., 1998. Development and mapping of microsatellite based markers in Eucalyptus. Theoretical and Applied Genetics 97:816-827; Brondani, R.P.V. 2001. Desenvolvimento, caracterização e mapeamento de marcadores microssatélites no gênero Eucalyptus. Tese de doutorado, Biologia Molecular, UnB).*

Table 1

Description of the twenty-five microsatellites markers recommended as molecular descriptors for the genetic profile determination in *Eucalyptus.* The size of the alleles bands located in base of pairs is indicated, as well as sequences of primers and linkage group in the genetic map.

Loci	Allele size (base pairs)	Sequence 5'-3' of direct primer	Sequence 5'-3'of reverse primer	Linkage Group
Embra01	100-145	gatagaactttcctatttgatcg	gtaggatttgatgtctgcaa	8
Embra02	103-148	cgtgacaccaggacattac	acaaatgcaaattcaaatga	11
Embra05	78-142	atgctggtccaactaagatt	tgagcctaaaagcccaac	5
Embra06	120-170	agagaattgctcttcatgga	gaaaagtctgcaaagtctgc	1
Embra10	110-152	gtaaagacatagtgaagacattcc	agacagtacgttctctagctc	10
Embra11	123-165	gcttagaatttgcctaaacc	gtaaaatccatgggcaag	1
Embra12	104-162	aggatttgtggggcaagt	gttccccattttcatgtcc	1
Embra15	90-125	tttgttggatgaggactt	caacatgttctccgaaaag	8
Embra16	110-165	caacgttccccttttcttc	atgttaggccaaacccag	1
Embra17	120-170	aggatactcgtgagagaagc	gtagatctgttctgcatgttg	9
Embra19	55-145	gacggttgatttcctgatt	gtggtgctcctctcctct	4
Embra23	118-145	ggttgtttcatcttttccatg	agcgaaggcaatgtgttt	10
Embra26	112-200	cccacaacaaaaggaaag	agaggtgttcgattcaattc	11
Embra27	100-170	ataaccacaccaatctgca	tatagctcgaacgctcaac	2
Embra28	180-300	caagacatgcatttcgtagt	actcttgatgtgacgagaca	6
Embra34	100-160	tcaaaaccctctctctcat	aataaacattttctttgaacaga	3
Embra37	115-165	cacctctccaaactacacaa	ctcctctctcttcaccattc	5
Embra42	115-170	gagtaaaaattggttttgagtg	ccctcttttcattttgtctt	7
Embra44	205-225	ggggtttgttctgcttag	caaaagagttcagctgtg	4
Embra46	90-130	gaagtcatcatctgtagattgc	acccattattctttgtgagc	7
Embra49	125-195	attattggttcatattgaaaacc	agatagagattgagtgagaccc	3
Embra51	95-200	gatgcattccttttttcc	cattctcttgcatctggac	6
Embra58	140-245	caccaactggtactatgaggat	ttggcttagggtagaacact	9
Embra63	175-230	catctggagatcgaggaa	gagagaaggatcatgcca	2
Embra72	118-170	ctggtcaacgtccgaaag	atgctgcagagggcataa	10

PART III

Description of the methods to be used

1. Extraction and quantification of DNA: The laboratory will utilize a procedure of extraction and quantification of genomic DNA from plant tissues (leaves, cambium, flowers, etc.). It is suggested that the protocol described by Ferreira & Grattapaglia (*Introdução ao uso de marcadores moleulares em análise genética, 1998. Terceira edição. Embrapa-SPI, pags. 121-130)* be used. The DNA must be quantified by electrophoresis in 0.8% agarose gel followed by ethidium bromide staining.

2. PCR (Polymerase chain reaction): The reactions of PCR for individual loci, are performed with 2 to 50 ng of genomic DNA; 1.5 mM; of Mg++; 0.25 μM of direct and reverse primers; 200 μM of each nucleotide; 0.2 mg/ml BSA; 1 x buffer PCR with 50 mM KCL; 10 mM TRIS-HCL pH 9.0; 0.1% Triton X-100; 1 polymerase unit of *Taq* DNA in a total volume of 15 μl. The PCR program in thermocycler apparatus is composed of an initial denaturation at 95° C for 4 minutes followed by 30 cycles of denaturation at 95° C for 1 minute and an extension at 65° C for 1 minute. There is a final extension step at 65° C for 10 minutes.

3. Polymorphism detection and genotype determination: To have a precise description of genetic profiles, the use of detection systems based upon fluorescence emissions in an automatic DNA sequencer is recommended, which allows for an exact definition of alleles in base pairs with a one base pair resolution. The primers for microsatellite loci must be marked with fluorchromes (blue (FAM); green (HEX); or yellow (NED)) and a specific spectrum filter, according with technology widely used in individual identification in human beings, animals and cultivated plants (*Fregeau, C.J. & Fourney, R.M. 1993 – DNA typing with fluorescently tagged short tamden repeats: a sensitive approach to human identification. Biotechniques 15(1): 100-119).* Each locus can be analyzed individually, or in "multiplex" combinations for simultaneous analyses of several loci. An internal standard marked with a fluorescent TAMRA or a red color ROX must be used for definition of fragment sizes. The amplified products are spotted on a polyacrylamide gel and separated in an automatic DNA sequencer.

4. Genetic interpretation and communication of descriptors: For each of the analyzed descriptor loci, the observed genotype should be identified and registered. The alleles will be visualized as peaks in the electropherogram and will be identified by their size in base pairs, estimated automatically by using an internal standard of known size (TAMRA or ROX). Genotypes should be described with the alleles identified in number of base pairs, rounded to the unit. The analysis should include, as control check, the DNA of a well characterized *Eucalyptus* clone, to be identified by the laboratory, to serve as a comparison of allele size in base pairs among laboratories or between different experiments within the same laboratory. When considered necessary, the probability of occurrence of the multi loci genetic profile could be estimated, based upon the classic principles of population genetics, assuming a Hardy-Wienberg equilibrium. This probability could be used to

establish significant statistical differences or the genetic identity between two samples, or even the existence of an essential derivation (VED).

[End of document]