UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

EUCALYPTUS *

UPOV Code:

(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 thirty-eighth session to be held in Seoul, Republic of Korea, from
September 12 to 16, 2005

Alternative Names:*

Botanical Name	English	French	German	Spanish
	Eucalyptus			

ASSOCIATED DOCUMENTS

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.

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* 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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ANNEX

1. <u>Subject of these Test Guidelines</u>

These Test Guidelines apply to all varieties of the species of the sections Transversaria, Maidenaria and Exsertaria of the sub-genus *Symphyomyrtus* of the genus *Eucalyptus*, cover only vegetatively propagated varieties.

2. <u>Material Required</u>

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, supplied from plants about 8 to 12 months old

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

10 young 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. <u>Method of Examination</u>

3.1 Number of Growing Cycles

The minimum duration of tests should normally be five (?)years. (3 years period is not consistent since there are some measurements done with 5 years. But all measures made on 4 and 5 years can be done with 3. So, should be chosen 3 or 4/5.)

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 observations of 4 and 5 years old plants should be made on plants of the clone selected during the breeding process (clonal tests). (Not consistent with 3.1, but may be used breeders tests?)

3.3.3   The observations of 0 to 3 years old plants should be carried out on plants obtained from the same clone being utilized simultaneously in the 4 and 5 years old tests.

3.3.4   Evaluation of characteristics should be made only once (one growing cycle).

3.4   Test Design

3.4.1   Each test should be designed to result in a total of at least 10 plants, which should be divided between 2 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

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.   <u>Grouping of Varieties and Organization of the Growing Trial</u>

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)   Young leaf: petiole (characteristic 2)
(b)   Primary branch (one year old): type of insertion in main stem (characteristic 13)
(c)   Inflorescence: number of buds per umbel (characteristic 23)
(d)   Fruit: shape (characteristic 31)
(e)   Tree: texture of basal bark on lower part (5 years it is very defined) (characteristic 35)

(d) and (e) may not be available if the variety does not flower.

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

(a)- (f) 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. (+)		**Plant: lignotuber**					
QL	**(a)**	absent					1
		present					9
2. (*) (+)		**Young leaf: petiole**					
QL	**(a)**	absent				*E. globulus*	1
		present				*E.grandis*	9
3. (*) (+)		**Young leaf: shape**					
PQ	**(a)**	linear					1
		narrow lanceolate					2
		medium lanceolate					3
		broad lanceolate					4
		ovate					5
		elliptic					6
		obovate					7
		cordate					8
		circular					9
		falcate					10
		oblique					11
		peltate					12

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4.		**Juvenile: waxiness**					
QN	**(a)**	absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
5. **(+)**		**Intermediate leaf: attitude of blade in the middle third of tree**					
QN	**(b)**	upwards					
		outwards	(Define what's intermediate". If it's related to the age of the tree or of the leaf.)				
		downwards					
6. **(+)**		**Intermediate leaf: petiole**					
QL	**(b)**	absent					1
		present					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
7. (*) (+)		**Intermediate leaf: shape**					
PQ	**(b)**	linear					1
		narrow lanceolate					2
		medium lanceolate					3
		broad lanceolate					4
		ovate					5
		elliptic					6
		obovate					7
		cordate					8
		circular					9
		falcate					10
		oblique					11
		peltate					12
8. (*)		**Intermediate leaf: anthocyanin coloration**					
QN	**(b)**	absent or very weak					1
		weak					3
		medium					5
		strong					7
9. (*)		**Intermediate leaf: glaucosity**					
QN	**(b)**	absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
10. **(+)**		**Stem: predominant collar of lower part**					
PQ	**(c)**	grey	(The word "rythidome"was not found in any dictionary. Does it exist in English? Could be better if replaced by "bark below the bark collar") Would it be interesting have a diagram as well)				1
		green					2
		brown					3
11.		**Stem: predominant color of middle part of stem (one year old)**					
PQ	**(c)**	cream					1
		grey					2
		green					3
		bluish green					4
		brown					5
12.		**Stem: glucosity of middle part on surface above the bark collar (one year)**					
QL	**(c)**	absent					1
		present					9
13. **(*)** **(+)**		**Primary branch (one year old): type of insertion in main stem**					
PQ	**(d)**	inverted "v"					1
	(e)	spherical					2

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
14.	**Color of wood on a primary branch (one year old) when bark is peeled away**					

(because the breeding is going toward the reddish color that indicate less lignin)

	English	français	deutsch	español	Example	Note
PQ	white					1
	green					2
	pink					3
	red					4
15.	**Stem: predominant color of middle part (3 years old)**					
PQ (e)	cream					1
	grey					2
	green					3
	bluish green					4
	brown					5

16 to 21: The leaf characteristics should indicate the location or even the age of the leaf (mature, intermediate or juvenile) that is linked also to the shape of the leaf

	English	français	deutsch	español	Example	Note
16.	**Leaf: attitude of adult leaf blade (3 years old)**					
QN (e)	upwards					1
	outwards					2
	downwards					3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
17. **(+)**		**Leaf: length of blade**					
QN	**(e)**	short					3
		medium					5
		long					7
18. **(+)**		**Leaf: width of blade**					
QN	**(e)**	narrow					3
		medium					5
		broad					7
19. **(*)** **(+)**		**Leaf: shape**					
PQ	**(e)**	linear					1
		narrow lanceolate					2
		medium lanceolate					3
		broad lanceolate					4
		ovate					5
		elliptic					6
		obovate					7
		cordate					8
		circular					9
		falcate					10
		oblique					11
		peltate					12

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
20.		**Leaf: intensity of green color of upper side relative to lower side**					
QN	**(e)**	lighter					1
		same					2
		darker					3
21.		**Leaf: glucosity of upper side**					
QN	**(e)**	absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
22. **(+)**		**Tree: age of first flowering**	**Could be added an explanation like**				
QN		early	Early: 1st year after planting in soil				3
		medium	Medium: 2-4 years				5
		late	Late: has not flowered by age 5				7

Note 1: in a plantation setting many eucalyptus clones do not flower.

Note 2: flowering late or "never" is a desirable characteristic being selected by some breeders because flowering may remove some of the potential biomass going into wood

Characteristics 23-34 are applied only for varieties that flower. The wording could be: "Only varieties that flower on early or medium age"

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
23.	**Inflorescence: number of buds per umbel**						
QL	three						1
	five						2
	seven						3
	more than seven						4
24.	**Inflorescence: peduncle**						
QL	absent						1
	present						9
25	**Inflorescence: length of peduncle**						
QN	short						3
	medium						5
	long						7
26.	**Inflorescence: shape of peduncle in cross section**						
PQ	rounded						1
	flattened						2

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
27.	**Flower bud: shape of opercule**					
(+)						
PQ	rostrate					1
	globose					2
	globose with a pointed tip					3
	flatted with a prominent pointed tip					4
	horn shaped					5
	elongated					6
	conical					7
28.	**Capsule: pedicel**					
QL	absent					1
	present					9
29.	**Fruit: length of pedicel related to the**					
QN	shorter					1
	equal					2
	longer					3
30.	**Fruit: size**					
QN	small					3
	medium					5
	large					7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
31. **(+)**	**Fruit: shape**					
PQ	conical					1
	pyriform					2
	cylindrical					3
	urceolate					4
	globose					5
	hemispherical					6
	campanulate					7
	ovoid					8
32. **(+)**	**Fruit: texture**					
QL	smooth					1
	rough					2
33. **(+)**	**Fruit: disk of opercule**					
QL	descending					1
	level					2
	ascending					3
34. **(+)**	**Fruit: valve**					
	inserted					1
	level					2
	extended					3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
35. (*) (+)		**Tree: texture of basal bark on lower part (5 years it is very defined)**	(can see at 3-4 years old, so why not at these ages?)				
PQ	(f)	smooth					1
		rough					2
		fibrous					3
36. (+)		**Tree: persistency of bark**					
QN	(f)	very low					1
		low					3
		medium					5
		high					7
		very high					9
37. (+)		**Plant: basic density of wood**					
PQ	(f)	low					1
		medium					2
		high					3

PROPOSED NEW CHARACTERISTICS:

(1)   Average stem diameter at this height could be another useful character very standard in breeding

(2)   Average distance between main branches (from 1,2 to 1,5 m in height). Branchiness is also a varietal characteristic: few-many.

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 plantlets with 3 to 4 months old.only to be observed on varieties with flower.
(b)   All observations should be made 6 months after planting.
(c)   All observations should be made 1 year after planting.
(d)   All observations should be made in one year old plants.
(e)   All observations should be made in 3 years old plants.
(f)   All observations should be made in 5 years old plants.

(a) Not consistent with 2.2 that indicates to start test with plants 8-12 months old. Could start younger (3-12 month old) and assess (a) characteristics just before planting in soil.
What's the difference between (c) and (d)?

(e) and (f) Not consistent with 3.3 that calls for tests to be carried out 3 years. You can do (f) characteristics at age 3-4.

8.2   Explanations for individual characteristics

Ad. 1: Plant: lignotuber
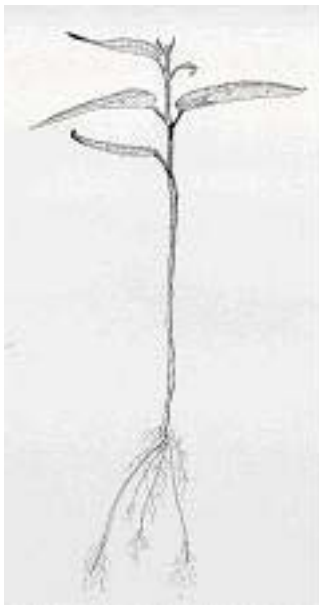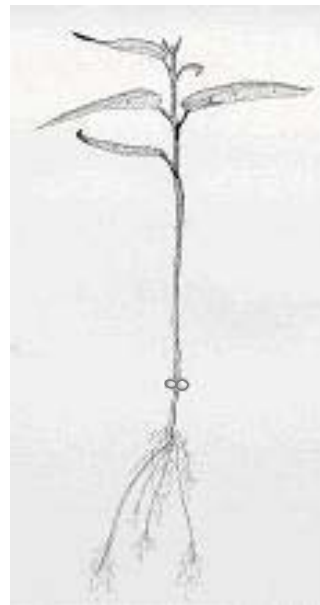
1   9
absent   present

Ad. 2: Young leaf: petiole
Ad. 6: Intermediate leaf: petiole

1	9
absent	present

Ad. 3:   Young leaf: shape
Ad. 7:   Intermediate leaf: shape
Ad. 19: Leaf: shape
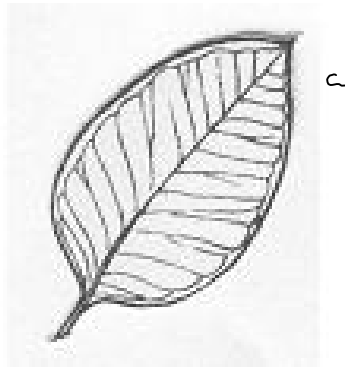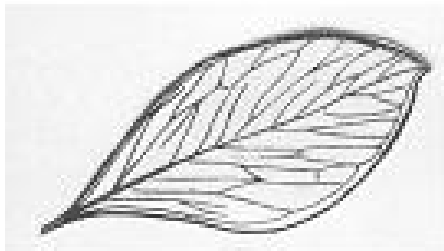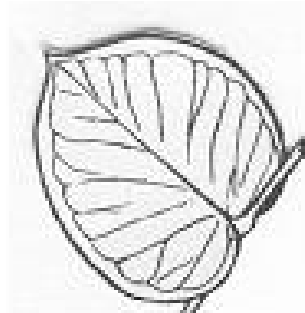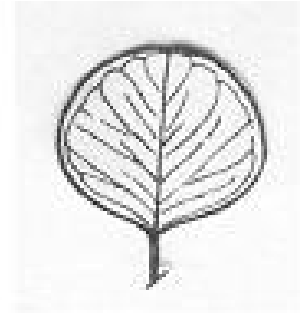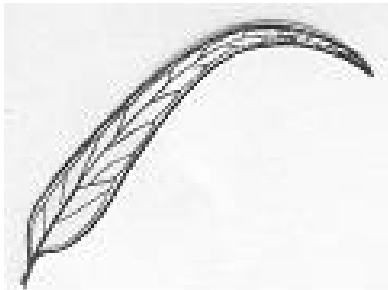
1	2	3
linear	narrow lanceolate	lanceolate
4	5	6
broad lanceolate	ovate	elliptic
7	8	9
obovate	cordate	circular
10	11	12
falcate	oblique	peltate

Ad. 5:   Intermediate leaf: attitude of blade in the middle third of tree

Ad. 16: Leaf: attitude of adult leaf blade (3 years old)

This characteristic should be observed with the branch positioned vertically.
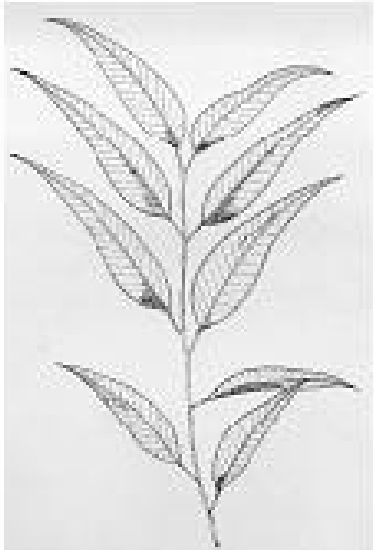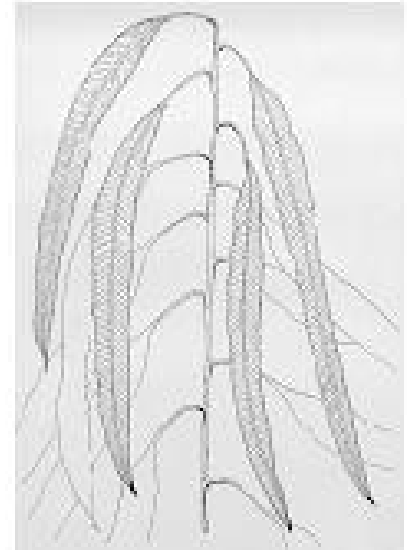
1	5	9
upwards	outwards	downwards

Ad. 13: Primary branch (one year old): type of insertion in main stem
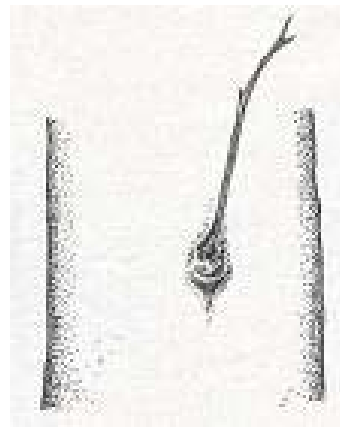
1	2
inverted "V"	spherical

Ad. 17: Leaf: length of blade

The length should be evaluated on the bigger leaf of a branch located in the beginning of the upper third of the crown in 3 year old plants. Maidenaria section should be evaluated in 5 year old plants.

Ad. 18: Leaf: width of blade

This evaluation should be performed on the same leaf selected for characteristic 17. The observation should be made in the widest part of the blade.
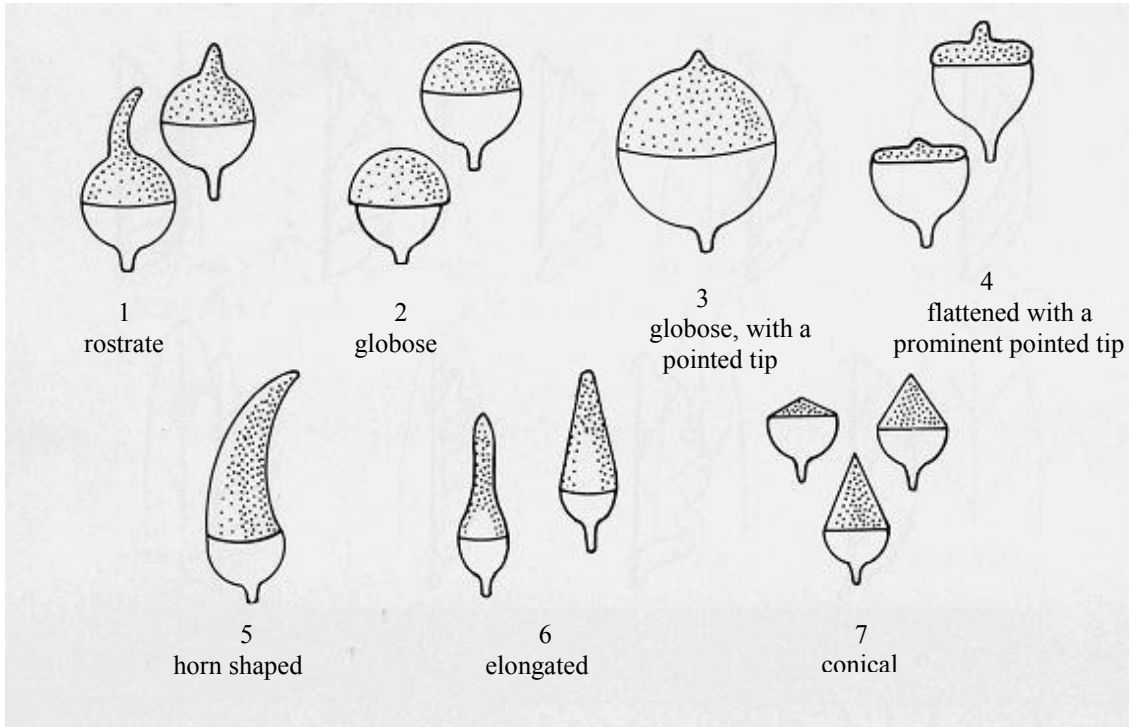
Ad. 24 to 27:

To evaluate these characteristics, inflorescence and flower bud parts are considered according to the drawing
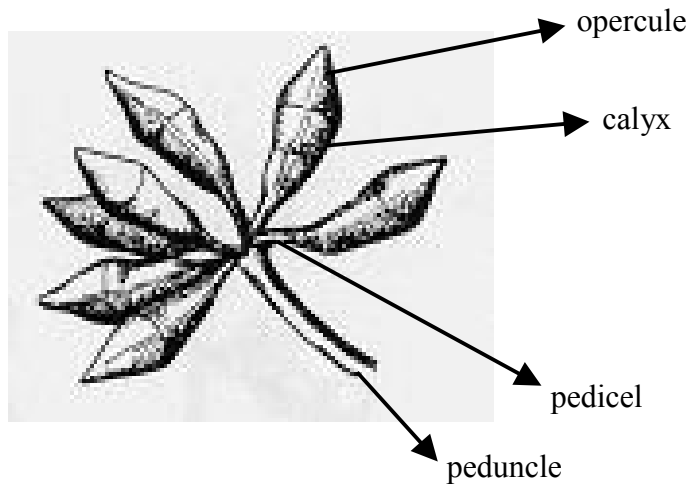
Ad. 24: Inflorescence: peduncle

| 1 | 2 |
| absent | present |
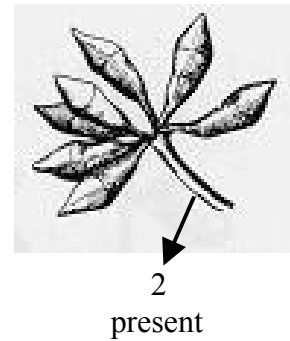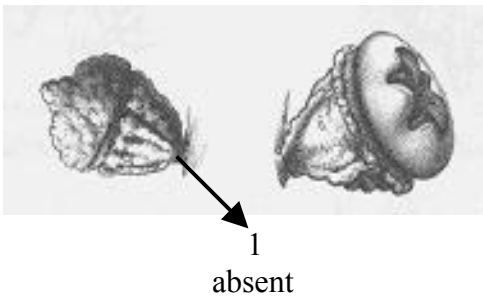
Ad. 27: Flowering bud: shape of opercule

| 1 rostrate | 2 globose | 3 globose, with a pointed tip | 4 flattened with a prominent pointed tip |

| 5 horn shaped | 6 elongated | 7 conical |

Ad. 28: Capsule: pedicel
Ad. 29: Fruit: length of pedicel related to the ....

To evaluate these characteristics, fruit parts are considered according to the drawing.

valves

opercule disk

pedicel
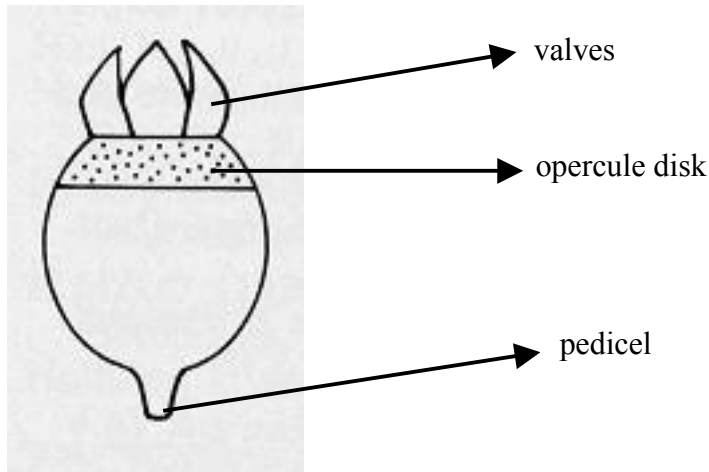
Ad. 30: Fruit: size

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

Ad. 31: Fruit: shape

1	2	3	4
conical	pyriform	cylindrical	urceolate

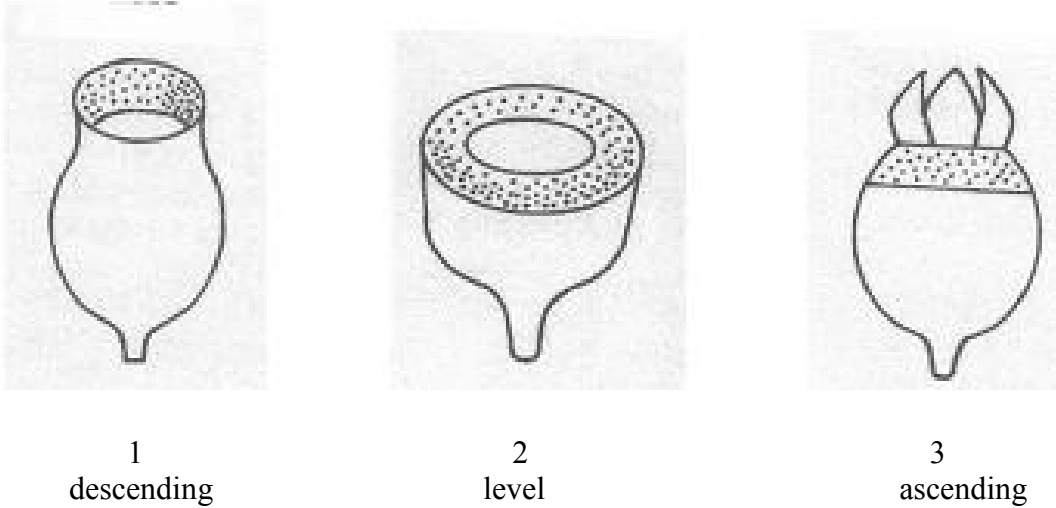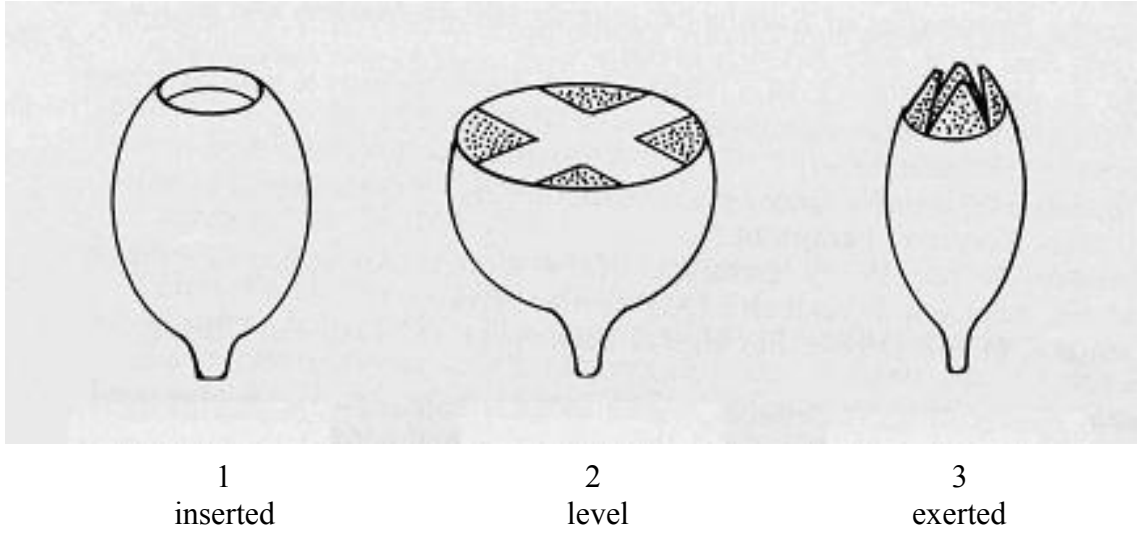| 5	6	7	8
globose	hemispherical	campanulate	ovoid
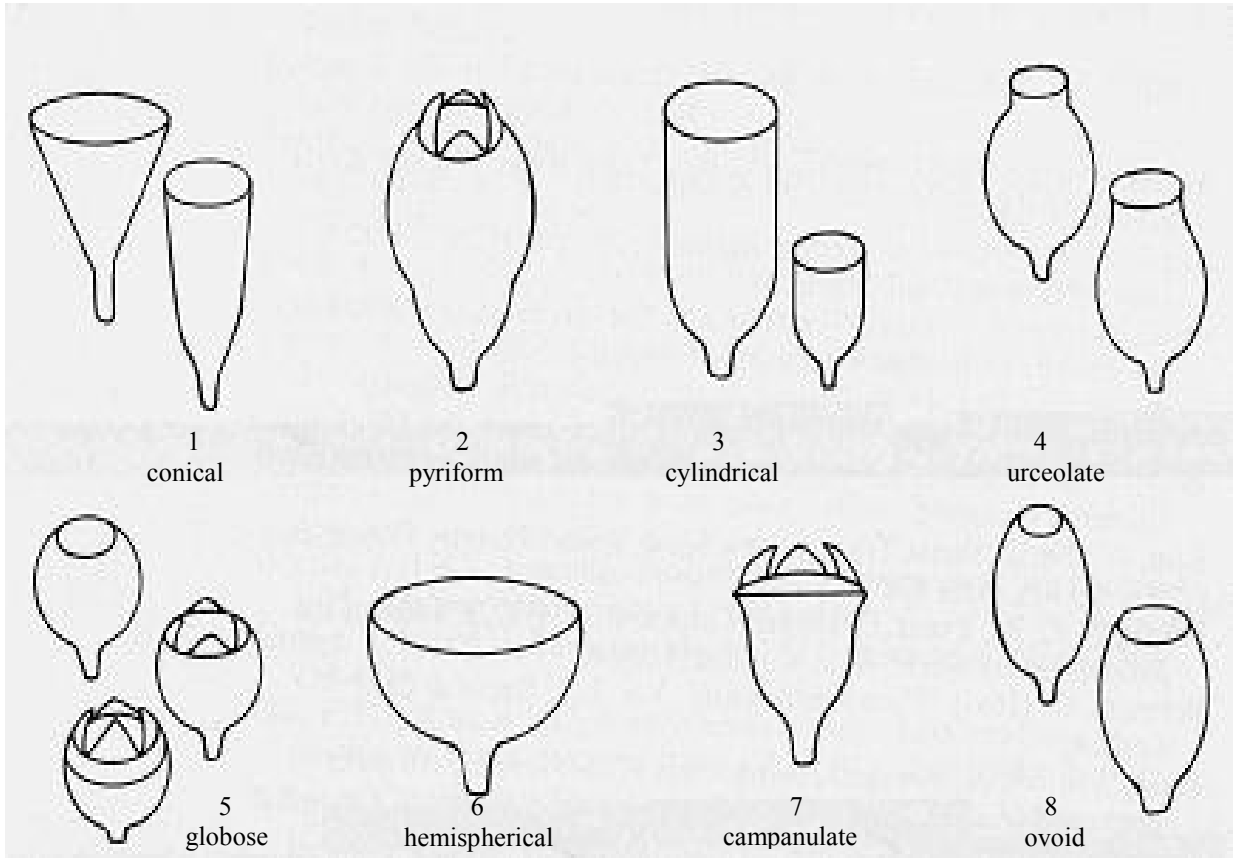
Ad. 32: Fruit: texture

The texture of the fruit should be observed during current year fruitage.
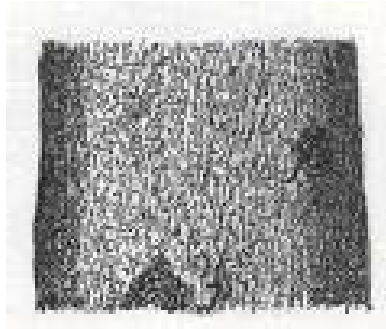
Ad. 33: Fruit: disk of opercule

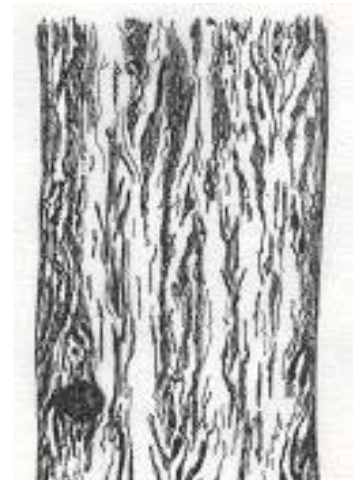| 1	2	3
descending	level	ascending

Ad. 34: Fruit: valves

1	2	3
inserted	level	exerted

Ad. 35: Tree: texture of basal bark on lower part (5 years it is very defined**)**

1	3	5
smooth	rough	fibrous

Ad. 36: Bark: persistency of bark

For evaluation purposes, the categories are defined as, approximately, the following persistency percentages related to the total area of the main stem:

<u>Ad. 37: Plant: basic density of wood</u>

Tree: basic density of wood (as for 35). The basic density should be evaluated through the hydrostatic balance methodology, according to TAPPI Norm #T258 om-94 (Technical Association of Pulp and Paper Industry). The samples, in a <u>wedge format</u>, must be drawn from a cross section of main stem at 1.3 m above the ground.

<u>If used a "wedge format" is the tree going to be cut down?</u>
Usually use a "radius" that can be taken by a cylindrical tool (core). So it is possible to measure non-destructively

9. <u>Literature</u>

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: Forest trees of Autralia. 4. ed. Melbourne: Nelson: CSIRO, 1994. 703 p.

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

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

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

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

10. <u>Technical Questionnaire</u>

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

<div align="center">

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

</div>

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)

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

3. Proposed denomination and breeder's reference

Proposed denomination
(if available)

Breeder's reference

#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)

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:

4.2 Method of propagating the variety

Example 2

4.2.1   Vegetative propagation

(a)   cuttings   [   ]

(b)   *in vitro* propagation   [   ]

(c)   other (state method)   [   ]

4.2.2   Seed   [   ]

4.2.3   Other   [   ]

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) **Plant: lignotuber**		
absent		1 [ ]
present		9 [ ]
5.2 (2) **Young leaf: petiole**		
absent	*E. globulus*	1 [ ]
present	*E.grandis*	9 [ ]
5.3 (13) **Primary branch (one year old): type of insertion in main stem**		
inverted "v"		1 [ ]
spherical		2 [ ]
5.4 (23) **Inflorescence: number of buds per umbel**		
three		1 [ ]
five		2 [ ]
seven		3 [ ]
more than seven		4 [ ]
5.5 (31) **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

5.6 (35) **Tree: texture of basal bark on lower part (5 years it is very defined)**

smooth 1 [ ]

rough 2 [ ]

fibrous 3 [ ]

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:

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

[Annex follows]

ANNEX

<u>Additional Useful Explanations</u>

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).*

<u>Table 1</u>

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	caacgttcccctttcttc	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	gatgcattcctttttttcc	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 Annex and of document]