

UPOV

**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA**

DRAFT

EUCALYPTUS

UPOV Code: 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 thirty-seven
session to be held in Hanover, Germany, from July 12 to 16, 2004*

Alternative Names: *

<i>Latin</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
	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 guidelines ("Test Guidelines") should be read in conjunction with document TG/1/3, "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants" (hereinafter referred to as the "General Introduction") and its associated "TGP" documents.

* These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of species within the sections *Transversaria*, *Maidenaria* and *Exsertaria* of the sub-genus *Symphomyrtus* 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, 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

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. Number of Growing Cycles

3.1 *Duration of Tests*

The minimum duration of tests should normally be conducted over three years.

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-year-old-plants should be made on plants of the clone selected during the breeding process (clonal tests).

3.3.3 The observations of 0- to 3-year-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 two 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 test, 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) Lignotuber (characteristic 1)
- (b) Young leaf: petiole (characteristic 2)
- (c) Primary branch (one-year-old): type of insertion in main stem (characteristic 13)
- (d) Inflorescence (simple): number of buds per umbel (characteristic 23)
- (e) Fruit: shape (characteristic 31)
- (f) Basal bark: texture (characteristic 35)

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 Section 6.1.2

(QL) Qualitative characteristic – see Section 6.3

(QN) Quantitative characteristic – see Section 6.3

(PQ) Pseudo-qualitative characteristic – see Section 6.3

(a) - (f) See Explanations on the Table of Characteristics in Chapter 8, Section 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8, Section 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				<i>E. grandis</i>	1
	present				<i>E. urophylla</i>	9
2. (*)	Young leaf: petiole					
(+)	(a) absent				<i>E. globulus</i>	1
QL	present				<i>E. grandis</i>	9
3. (*)	Young leaf: shape					
(+)	(a) linear					1
PQ	narrow lanceolate					2
	medium lanceolate					3
	broad lanceolate					4
	ovate					5
	elliptic					6
	obovate					7
	cordate					8
	orbicular					9
	falcate					10
	oblique					11
	peltate					12
4.	Young leaf: waxiness					
QN	(a) absent or very weak					1
	weak					3
	medium					5
	strong					7
	very strong					9

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielsorten/ Variedades ejemplo	Note/ Nota
5. (+)	Intermediate leaf: attitude of blade					
QN	(b) erect					1
	semi erect					3
	horizontal					5
	semi drooping					7
	drooping					9
6. (+)	Intermediate leaf: petiole					
QL	(b) absent					1
	present					9
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
	orbicular					9
	falcate					10
	oblique					11
	peltate					12

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
8. (*)	Intermediate leaf: anthocyanin coloration					
QN	(b)					1
					absent or very weak	
					weak	3
					medium	5
					strong	7
9. (*)	Intermediate leaf: waxiness					
QN	(b)					1
					absent or very weak	
					weak	3
					medium	5
					strong	7
					very strong	9
10.	Stem: predominant color of rythidome					
PQ	(c)					1
					grey	
					green	2
					brown	3
11.	Stem: predominant color of surface above rythidome					
PQ	(c)					1
					cream	
					grey	2
					green	3
					bluish green	4
					brown	5

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
12.	Stem: wariness on surface above rythidome					
QL	(c)	absent				1
		present				9
13. (*) (+)	Primary branch (one year old): type of insertion in main stem					
PQ	(d)	inverted “v”				1
		spherical				2
14. (*)	Stem: predominant color of rythidome					
PQ	(e)	grey				1
		green				2
		brown				3
15.	Stem: predominant color of surface above rythidome					
PQ	(e)	cream				1
		grey				2
		green				3
		bluish green				4
		brown				5
16.	Leaf: attitude of blade					
QN	(e)	erect				1
		semi erect				3
		horizontal				5
		semi drooping				7
		drooping				9

	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
		orbicular				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					
PQ						
	(e) darker on lower surface					1
	same intensity on both surfaces					2
	darker on upper surface					3
21.	Leaf: waxiness					
QN	(e)					
	absent or very weak					1
	weak					3
	medium					5
	strong					7
	very strong					9
22.	Tree: time of first flowering					
QN	early					4
	medium					5
	late					7
23.	Inflorescence (simple): number of buds per umbel					
QL						
	one					1
	three					2
	five					3
	seven					4
	more than seven					5

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24. (+)	Inflorescence: peduncle					
QL	absent					1
	present					9
25. (+)	Inflorescence: length of peduncle					
QN	short					3
	medium					5
	long					7
26. (+) PQ	Inflorescence: shape of peduncle (cross section)					
	rounded					1
	flattened					2
27. (+)	Flowering 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. (+)	Fruit: pedicel					
QL	absent					1
	present					9

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
29. (+)	Fruit: length of pedicel relative to the length of the fruit					
QN	shorter					3
	equal					5
	longer					7
30. (+)	Fruit: size					
QN	Small					3
	medium					5
	large					7
31. (+)	Fruit: shape					
PQ	conical					1
	pyriform					2
	cylindrical					3
	urceolate					4
	globose					5
	hemispherical					6
	campanulate					7
	ovoid					8
33. (+)	Fruit: disk of opercule					
PQ	descending					1
	level					2
	ascending					3

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
34. (+)	Fruit: valve					
PQ	inserted					1
	level					2
	exerted					3
35. (*)	Basal bark: texture					
(+)	(f) smooth					1
PQ	rough					2
	fibrous					3
36. (+)	Bark: persistency of the rough or fibrous bark (as for 35)					
QN	(f) very low					1
	low					3
	medium					5
	high					7
	very high					9
37. (+)	Plant: basic density of wood					
PQ	(f) up to 0,4 g/cm ³					1
	from 0,41 to 0,5 g/cm ³					2
	above 0,5 g/cm ³					3

8. Explanations on the Table of Characteristics

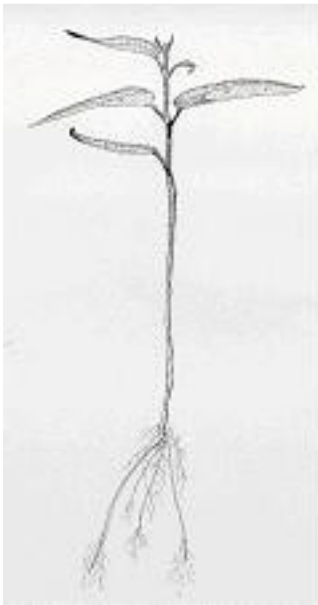
8.1 *Explanations covering several characteristics*

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

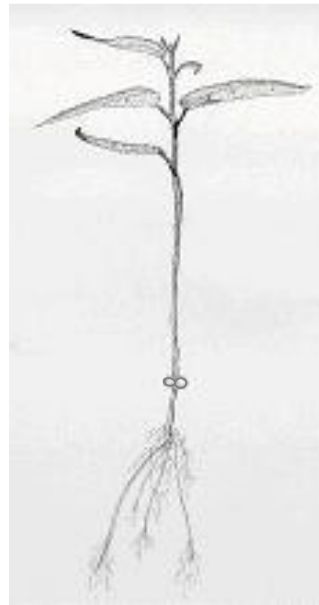
- (a) Observations to be made on plantlets which are 3 to 4 months old.
- (b) Observations to be made 6 months after planting.
- (c) Observations to be made 1 year after planting.
- (d) Observations to be made on one-year-old plants.
- (e) Observations to be made on 3-year-old plants.
- (f) Observations to be made on 5-year-old plants.

8.2 *Explanations for individual characteristics*

Ad. 1: Plant: lignotuber

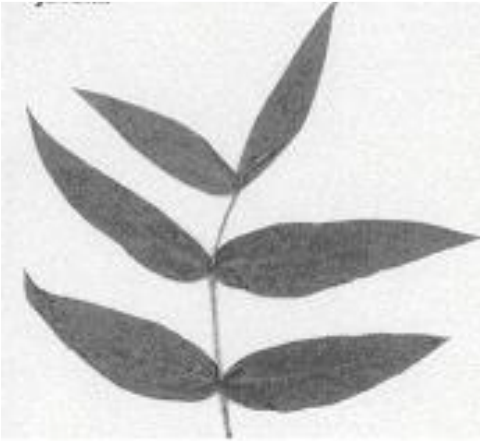


1
absent



9
present

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

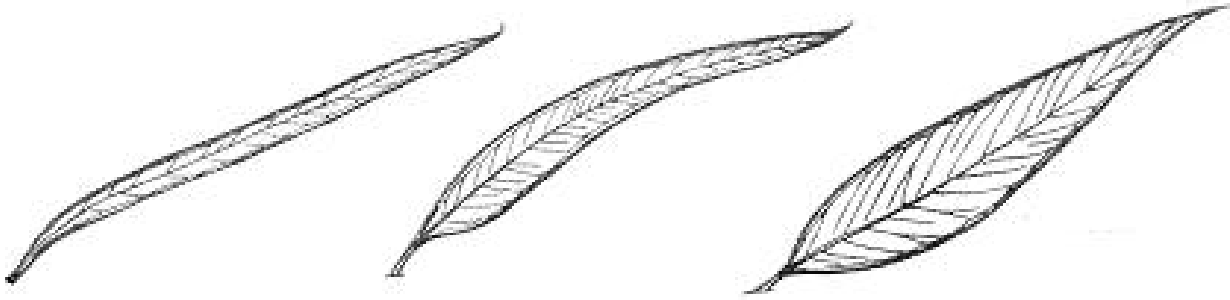


1
absent



9
present

Ad. 3 / Ad. 7 / Ad. 19. Leaf: shape



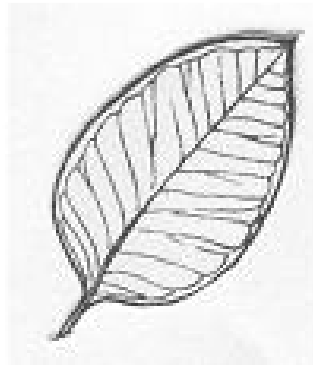
1
linear

2
narrow lanceolate

3
lanceolate



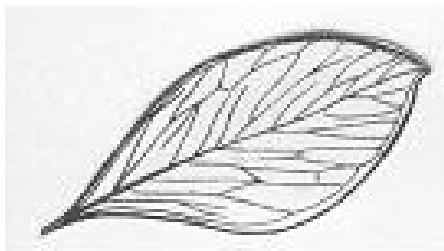
4
broad lanceolate



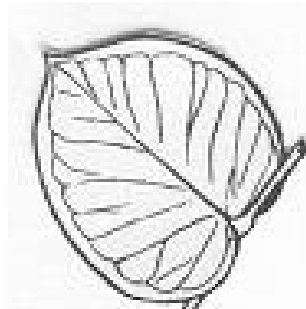
5
ovate



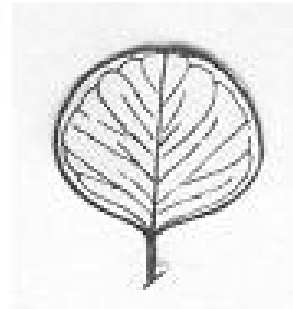
6
elliptic



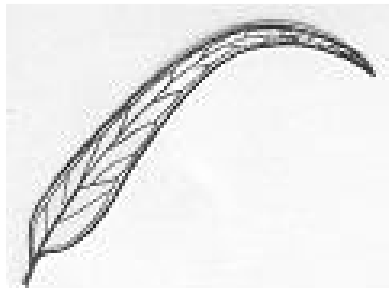
7
obovate



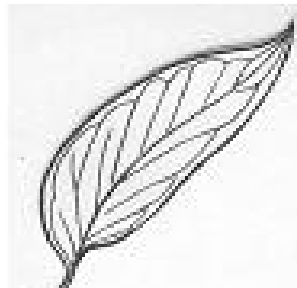
8
cordate



9
orbicular



10
falcate



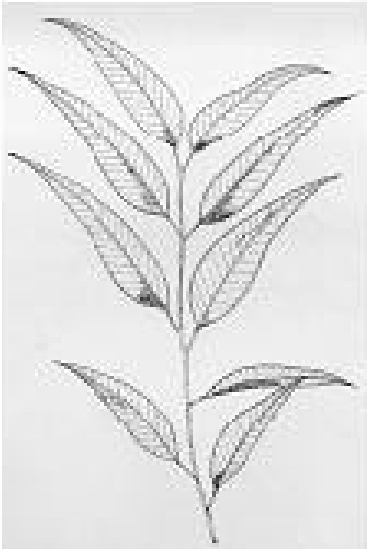
11
oblique



12
peltate

Ad. 5 / Ad. 16: Leaf: attitude of blade

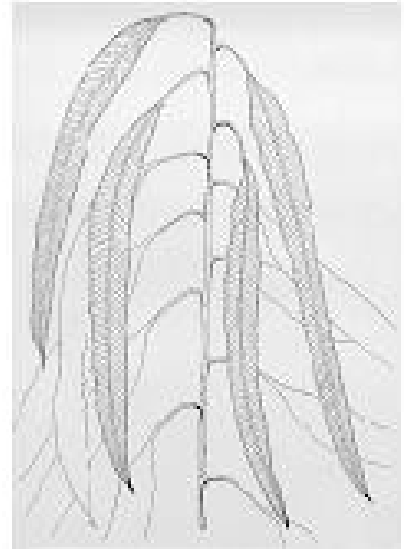
This characteristic should be observed with branch vertically positioned.



1
erect



5
horizontal

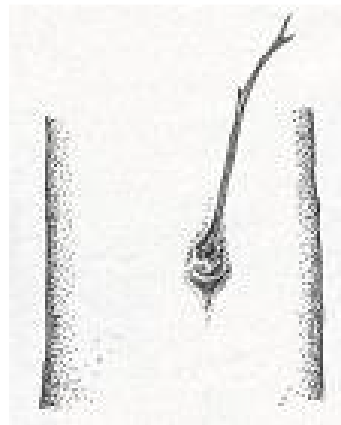


9
drooping

Ad. 13: Primary branch (one year old): type of insertion in main stem



1
inverted "V"



2
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 years old plants. Maidenaria section should be evaluated in 5 years old plants.

Ad. 18: Leaf: width of blade (in 3 years old plants)

This evaluation should be performed over the same leaf selected for characteristic 17. The observation should be made in the wider part of the blade.

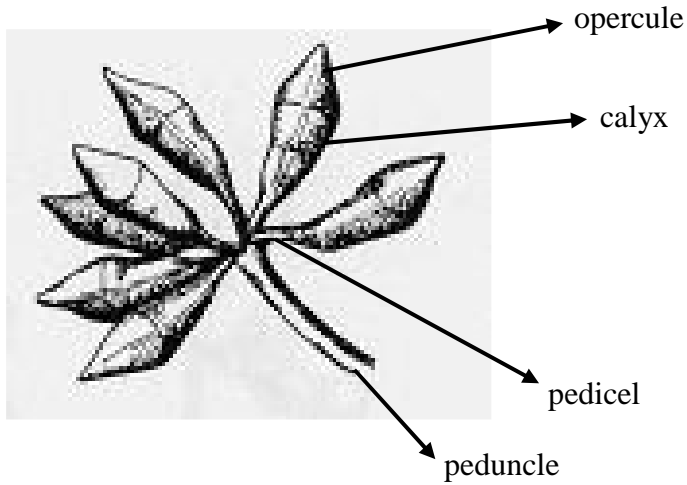
Ad. 24 Inflorescence: peduncle

Ad. 25 Inflorescence: length of peduncle

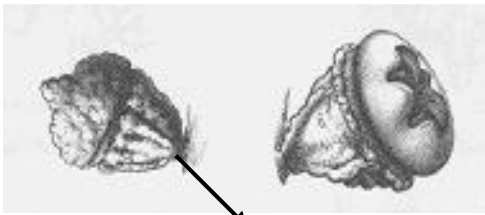
Ad. 26 Inflorescence: shape of peduncle

Ad. 27 Flowering bud: shape of opercule

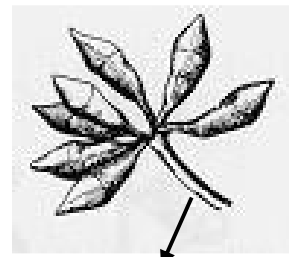
To evaluate these characteristics, inflorescence and flower bud parts are considered according to the drawing



Ad. 24: Inflorescence: peduncle

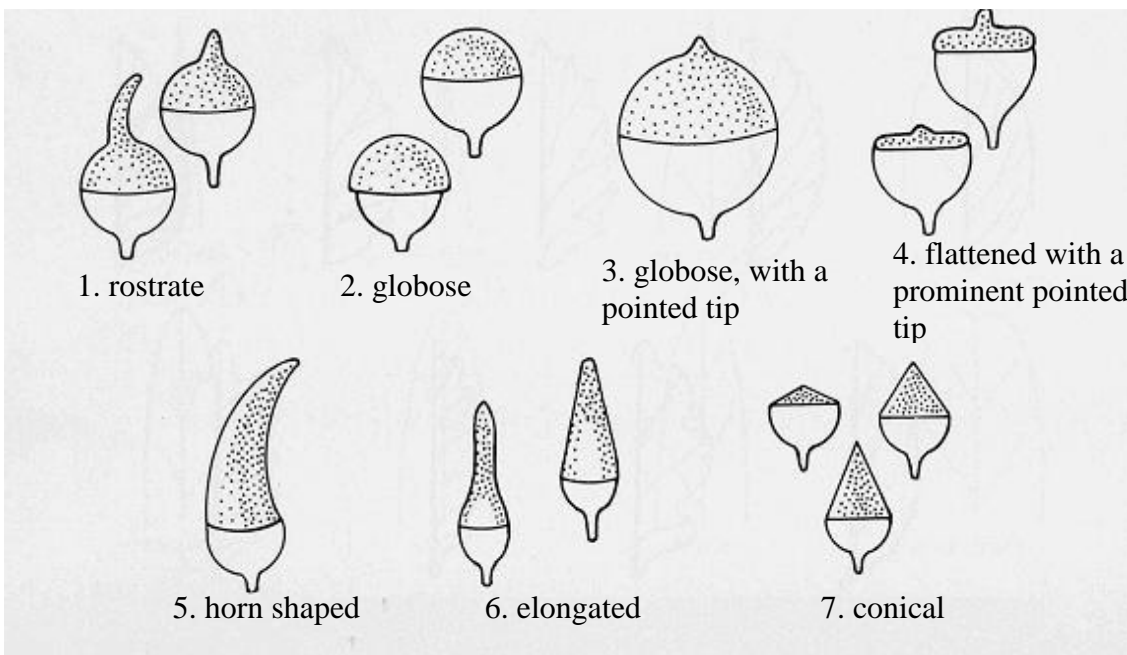


1
absent



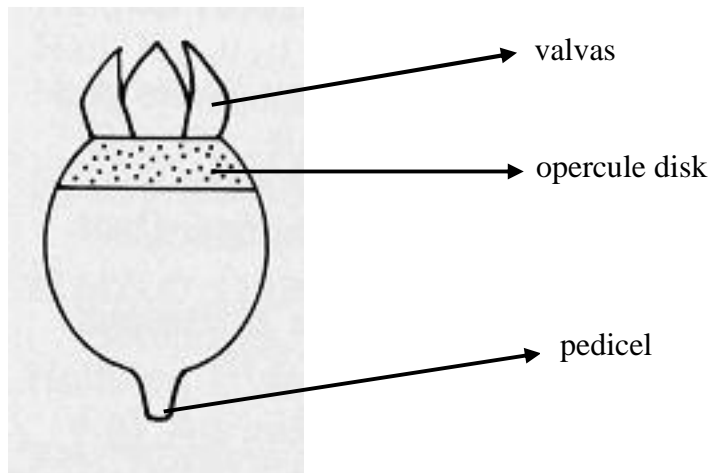
9
present

Ad. 27: Flowering bud: shape of opercule



Ad. 28 Fruit: pedicel / Ad. 29 Fruit: length of pedicel relative to the length of the fruit

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

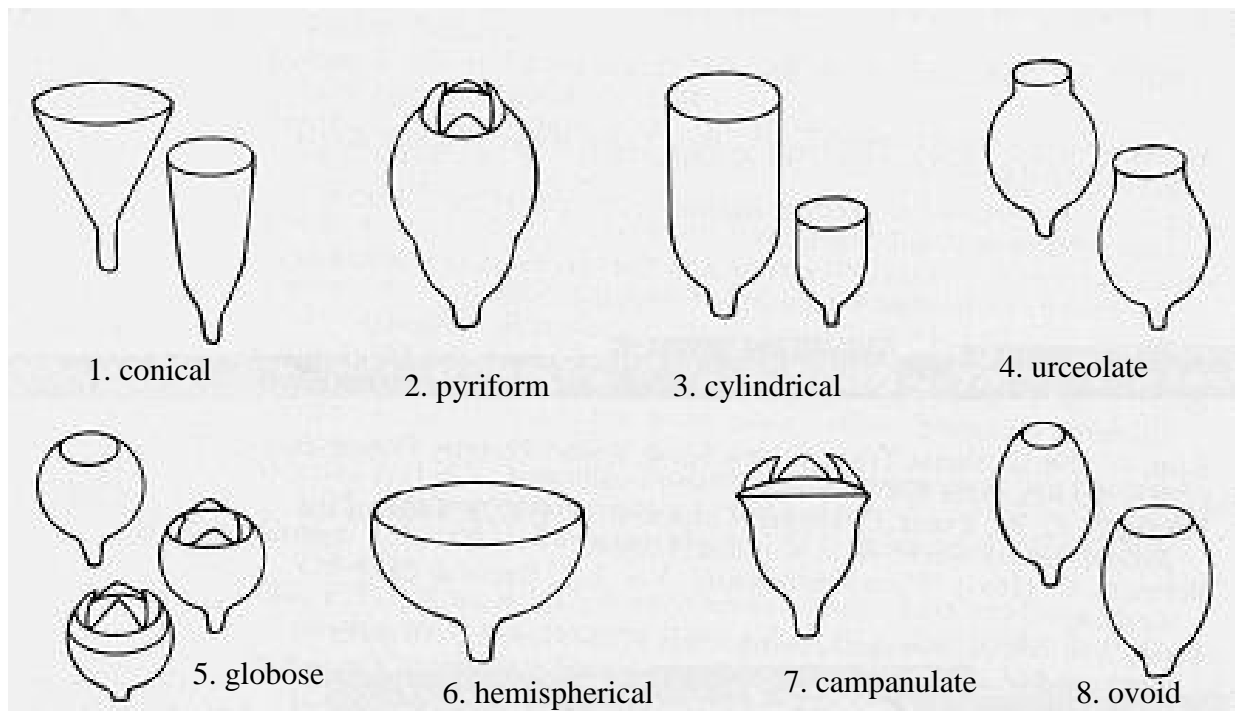


Ad. 30 Fruit: size

To be evaluated (measured) on its larger diameter. It should be considered as:

- (3) small : up to 0.5 cm
- (5) medium : approximately between 0.5 and 1.0 cm
- (7) large : above 1.0 cm

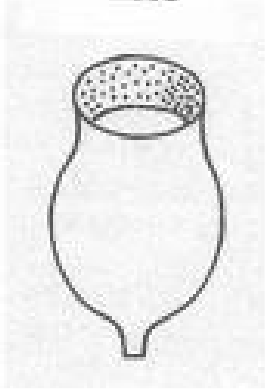
Ad. 31. Fruit: shape



Ad. 32: Fruit: texture

To be observed during current year fruitage

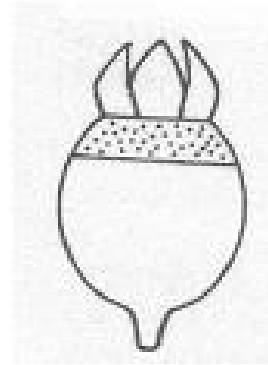
Ad. 33: Fruit: disk of opercule



1
descending

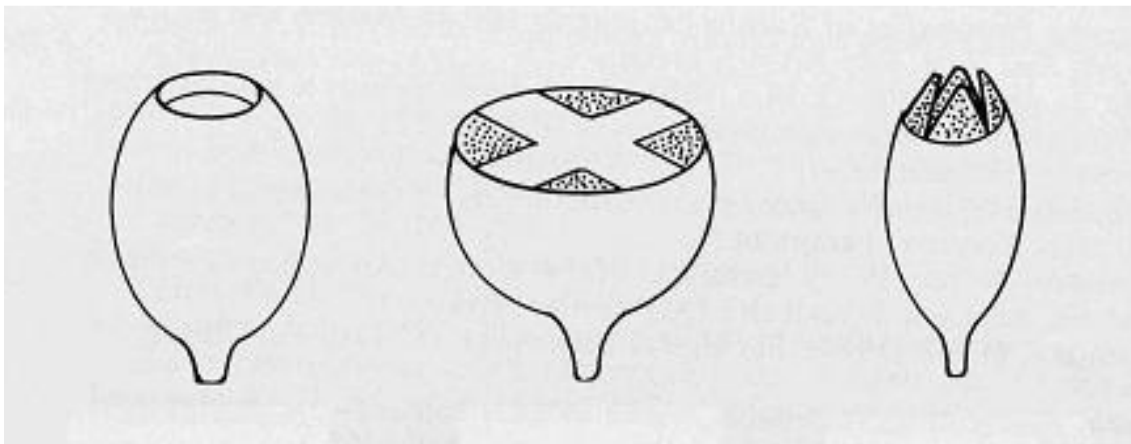


2
level



3
ascending

Ad. 34: Fruit: valve



1
inserted



2
level

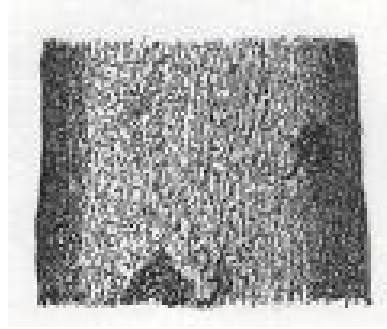


3
exerted

Ad. 35: Bark at base: texture (in 5 years old tree)



1
smooth



2
rough



3
fibrous

Ad. 36: Bark: persistency of rough or fibrous bark (as for 35).

For evaluation purpose, it is considered, approximately, the following persistency percentages related to the total bark area:

(1) very low	up to 20%
(3) low	between 20 and 40%
(5) medium	between 40 and 60%
(7) high	between 60 and 80%
(9) very high	above 80%

Ad. 37

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 wedge format, must be drawn from a cross section of main stem at 1.3 m above the ground.

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. Forest trees of Australia. 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. 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	<input type="text" value="Eucalyptus: Symphymyrtus"/> Section: Transversaria – Exsertaria - Maidenaria	
1.2 Species (please indicate species or hybrids)	<input type="text"/>	
2. Applicant		
Name	<input type="text"/>	
Address	<input type="text"/>	
Telephone No.	<input type="text"/>	
Fax No.	<input type="text"/>	
E-mail address	<input type="text"/>	
Breeder (if different from applicant)	<input type="text"/>	

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

(i) 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

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:
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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 Plant: ligno-tuber		
(1) absent		1[]
present		9[]
5.2 Young leaf: petiole		
(2) absent		1[]
present		9[]
5.3 Primary branch (one year old): type of insertion in main stem		
(13) inverted 'v'		1[]
spherical		2[]
5.4 Inflorescence (simple): number of buds per umbel		
(23) one		1[]
three		2[]
five		3[]
seven		4[]
more than seven		5[]
5.5 Fruit: shape		
(31) Conical		1[]
pyriform		2[]
cylindrical		3[]
urceolate		4[]
Globose		5[]
hemispherical		6[]
campanulate		7[]
ovoid		8[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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5.6 Basal bark: texture	
(36) 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
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<i>Example</i>

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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#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:
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9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- | | | |
|---|---------|--------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma) | Yes [] | No [] |
| (b) Chemical treatment (e.g. growth retardant, pesticide) | Yes [] | No [] |
| (c) Tissue culture | Yes [] | No [] |
| (d) Other factors | Yes [] | No [] |

Please provide details of 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 micro satellites 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 the determination of the genetic profile of a sample, twenty-five loci microsatellites are recommended, according to the table below, to allow a standardization of the genetic profiles generated. Two molecular markers are recommended for each one of the eleven linkage groups, corresponding to the eleven chromosomes of *Eucalyptus*, and other three additional markers. The analyst can utilize the number of loci considered necessary for his/her specific situation, looking first for genetically independent markers or, in other words, for different groups of linkage. However, it is important for the user to utilize only recommended markers only, allowing comparisons among several testing laboratories. Higher the number of loci used, bigger the power of discrimination of the , 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	gatagaactttctatttgatcg	gtaggatttgatgtctgcaa	8
Embra02	103-148	cgtgacaccaggacattac	acaatgcaaattcaaatga	11
Embra05	78-142	atgctggtccaactaagatt	tgagcctaaaagcccaac	5
Embra06	120-170	agagaattgctcttcatgga	gaaaagtctgcaaagtctgc	1
Embra10	110-152	gtaaagacatagtggaagacattcc	agacagtacgttctctagctc	10
Embra11	123-165	gcttagaatttgccataaac	gtaaaatccatgggcaag	1
Embra12	104-162	aggatttggtgggcaagt	gttcccattttcatgtcc	1
Embra15	90-125	tttgttgatgaggactt	caacatgttctccgaaaag	8
Embra16	110-165	caacgttcccctttcttc	atgttaggccaaaccag	1
Embra17	120-170	aggatactgtgagagaagc	gtagatctgttctgcatgtg	9
Embra19	55-145	gacggttgatttctgatt	gtggtgctctctctctct	4
Embra23	118-145	ggttgtttcatcttttccatg	agcgaaggcaatgtgttt	10
Embra26	112-200	cccacaacaaaaggaaaag	agaggtgttcgattcaattc	11
Embra27	100-170	ataaccacaccaaatctgca	tatagctcgaacgtcaac	2
Embra28	180-300	caagacatgcatttctgtagt	actcttgatgtgacgagaca	6
Embra34	100-160	tcaaaacctctctctcat	aataaacattttctttgaacaga	3
Embra37	115-165	cacctctccaaactacacaa	ctctctctcttaccattc	5
Embra42	115-170	gagtaaaaattggtttgagtg	ccctctttcattttgtctt	7
Embra44	205-225	ggggtttgttctgcttag	caaaagagttcagctgtg	4
Embra46	90-130	gaagtcacatctgtagattgc	accattattctttgtgagc	7
Embra49	125-195	attattggttcatattgaaaacc	agatagagattgagtgagacc	3
Embra51	95-200	gatgcattccttttttcc	cattctcttgcactggac	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 moleculares 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 (Polimerase 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 polimerase unit of *Taq* DNA in a total volume of 15 µl. The PCR program in thermocycler apparatus is composed of an initial denaturalization at 95° C for 4 minutes followed by 30 cycles of denaturalization at 95° C for 1 minute and na extension at 65° C for 1 minute. There is a final extension step at 65° C for 10 minutes.
3. Polymorphism's detection and genotype determination: To have a precise description of genetic profiles, the use of detection systems based upon fluorecence 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 microsatellites loci, must be marked with fluorchromes (blue (FAM); green (HEX); or yellow (NED)) and a specific filter of specter, according with a 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 loci 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 poliacrylamid gel and separated in an automatic DNA sequencer.
4. Genetic interpretation and communication of descriptors: For each one 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 alleles size in base pairs of bases among laboratories or between different experiments within the same laboratory. When considered necessary, it could be estimated the probability of occurrence of the multi loci genetic profile, based upon the classic principles of population genetics, assuming 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).