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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

HEMP

UPOV Code: CANNB_SAT

Cannabis sativa L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by an expert from the Netherlands

to be considered by the

*Enlarged Editorial Committee at its meeting
to be held in Geneva, on January 11 and 12, 2012*

Alternative Names:*

<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
<i>Cannabis sativa</i> L.	Hemp	Chanvre	Hanf	Cáñamo

The purpose of these guidelines (“Test Guidelines”) is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

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

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Cannabis sativa* L.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed or young, non-flowering plants in pots, of sufficient size and with sufficient development to express all the characteristics of the variety in the first growing cycle.

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

Vegetatively propagated varieties: 50 young plants.

Seed-propagated varieties: 500 grams of seed.

In the case of seed, the seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 *Number of Growing Cycles*

The minimum duration of tests should normally be two independent growing cycles.

3.2 *Testing Place*

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness".

3.3 *Conditions for Conducting the Examination*

3.3.1 The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.3.2 The optimum stage of development for the assessment of each characteristic is indicated by a number in the second column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.

3.4 *Test Design*

3.4.1 In the case of seed-propagated varieties, each test should be designed to result in a total of at least 200 plants, which should be divided between at least 2 replicates.

3.4.2 In the case of vegetatively propagated varieties, each test should be designed to result in a total of at least 40 plants.

3.4.3 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 *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.

4.1.4 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 20 plants or parts taken from each of 20 plants and any other observations made on all plants in the test, disregarding any off-type plants.

4.1.5 Method of Observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the second column of the Table of Characteristics (see document TGP/9 “Examining Distinctness”, Section 4 “Observation of characteristics”):

MG: single measurement of a group of plants or parts of plants

MS: measurement of a number of individual plants or parts of plants

VG: visual assessment by a single observation of a group of plants or parts of plants

VS: visual assessment by observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)

“Visual” observation (V) is an observation made on the basis of the expert’s judgment. For the purposes of this document, “visual” observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. color charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, “G” provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness.”

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

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 Seed-propagated varieties: the assessment of uniformity of seed-propagated varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.

4.2.3 Vegetatively propagated varieties: for the assessment of uniformity of vegetatively propagated varieties, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 40 plants, 2 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 further examined by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the initial 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.

The following have been agreed as useful grouping characteristics:

- (a) Time of male flowering (characteristic 11)
- (b) Inflorescence: THC content (characteristic 13)
- (c) Plant: proportion of hermaphrodite plants (characteristic 14)
- (d) Plant: proportion of female plants (characteristic 15)
- (e) Plant: proportion of male plants (characteristic 16)
- (f) Plant: natural height (characteristic 17)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 "Examining Distinctness".

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*

6.2.1 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.

6.2.2 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	Note
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9

6.2.3 Further explanation of the presentation of states of expression and notes is provided in document TGP/7 "Development of Test Guidelines".

6.3 *Types of Expression*

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 *Example Varieties*

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.5 *Legend*

(*) Asterisked characteristic – see Chapter 6.1.2

QL Qualitative characteristic – see Chapter 6.3

QN Quantitative characteristic – see Chapter 6.3

PQ Pseudo-qualitative characteristic – see Chapter 6.3

MG, MS, VG, VS – see Chapter 4.1.5

C Additional test in greenhouse

(a), (b) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.2

0003, etc. Growth stage – see Chapter 8.3

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. 0003 VG (+)	Cotyledon: shape	Cotylédon : forme	Keimblatt: Form	Cotiledón: forma		
QN C	narrow obovate	obovale étroit	schmal verkehrt eiförmig	oboval estrecha	Fibrimon	1
	medium obovate	obovale moyen	mittel verkehrt eiförmig	oboval media	Epsilon 68	2
	broad obovate	obovale large	breit verkehrt eiförmig	oboval ancha	Futura 75	3
2. 0003 VG	Cotyledon: color	Cotylédon : couleur	Keimblatt: Farbe	Cotiledón: color		
PQ C	yellow	jaune	gelb	amarillo	Chamaeleon	1
	light green	vert clair	hellgrün	verde claro	Fedora 17	2
	medium green	vert moyen	mittelgrün	verde medio	Ferimon	3
	dark green	vert foncé	dunkelgrün	verde oscuro	Dioica 88	4
3. 0003 VG	Hypocotyl: intensity of anthocyanin coloration	Hypocotyle : intensité de la pigmentation anthocyanique	Hypocotyl: Intensität der Anthocyanfärbung	Hipocotilo: intensidad de la pigmentación antociánica		
QN C	weak	faible	gering	débil	Usó 31	3
	medium	moyenne	mittel	media	Dioica 88	5
	strong	forte	stark	fuere	KC Dora	7
4. 1006 VG	Plant: anthocyanin coloration of crown	Plante : pigmentation anthocyanique de la couronne	Pflanze: Anthocyanfärbung der Krone	Planta: pigmentación antociánica de la corona		
QN	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil		1
	weak	faible	gering	débil	Felina 32	3
	medium	moyenne	mittel	media	Epsilon 68	5
	strong	forte	stark	fuerte	Finola	7
	very strong	très forte	sehr stark	muy fuerte		9

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
5.	VG	Leaf: intensity of green color	Feuille : intensité de la couleur verte	Blatt: Intensität der Grünfärbung	Hoja: intensidad del color verde	
QN	(a)	light	claire	hell	ligero	Chamaeleon 1
		medium	moyenne	mittel	medio	Fedora 17 2
		dark	foncée	dunkel	oscuro	Epsilon 68 3
6.	MS	Leaf: length of petiole	Feuille : longueur du pétiole	Blatt: Länge des Blattstiels	Hoja: longitud del pecíolo	
QN	(a)	short	court	kurz	corto	Santhica 27 1
	(b)	medium	moyen	mittel	medio	Fedora 17 2
		long	long	lang	largo	Ermes 3
7.	VG	Hoja: anthocyanin coloration of petiole	Feuille : pigmentation anthocyanique du pétiole	Blatt: Anthocyanfärbung des Blattstiels	Hoja: pigmentación antocianica del pecíolo	
QN	(a)	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Fibrol 1
	(b)	weak	faible	gering	débil	Ruby 2
		medium	moyenne	mittel	media	Dioica 88 3
		strong	forte	stark	fuerte	Epsilon 68 4
		very strong	très forte	sehr stark	muy fuerte	Finola 5
8.	MS/ (*) (+)	Leaf: number of leaflets	Feuille : nombre de folioles	Blatt: Anzahl Blattfiedern	Hoja: número de folíolos	
QN	(a)	few	faible	gering	bajo	Ermes 1
	(b)	medium	moyen	mittel	medio	Epsilon 68 2
		many	élevé	groß	alto	Kompolti 3
9.	MS	Central leaflet: length	Foliole centrale : longueur	Mittlere Blattfieder: Länge	Folíolo central: longitud	
QN	(a)	short	courte	kurz	corto	Santhica 27 3
	(b)	medium	moyenne	mittel	medio	Epsilon 68 5
		long	longue	lang	largo	Kompolti 7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota	
10.	MS	Central leaflet: width	Foliole centrale : largeur	Mittlere Blattfieder: Breite	Folíolo central: anchura		
QN	(a)	narrow	étroite	schmal	estrecho	Santhica 27	3
	(b)	medium	moyenne	mittel	medio	Dioica 88	5
		broad	large	breit	ancho	Kompolti	7
11.	MG	Time of male flowering	Époque de floraison mâle	Zeitpunkt der männlichen Blüte	Época de floración masculina		
	(*) (+)						
QN		very early	très précoce	sehr früh	muy temprana	Finola	1
		early	précoce	früh	temprana	Santhica 27	3
		medium	moyenne	mittel	media	Dioica 88	5
		late	tardive	spät	tardía	Futura 75	7
		very late	très tardive	sehr spät	muy tardía	Kompolti	9
12.	2102 2304 VG	Inflorescence: anthocyanin coloration of male flowers	Inflorescence : pigmentation anthocyanique des fleurs mâles	Blütenstand: Anthocyanfärbung der männlichen Blüten	Inflorescencia: pigmentación antociánica de las flores masculinas		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Kompolti	1
		weak	faible	gering	débil	Beniko	3
		medium	moyenne	mittel	media	Usó 31	5
		strong	forte	stark	fuerte	Ermes	7
		very strong	très forte	sehr stark	muy fuerte	Finola	9
13.	MG	Inflorescence: THC content	Inflorescence : teneur en THC	Blütenstand: THC-Gehalt	Inflorescencia: contenido en THC		
	(*) (+)						
QN		absent or very low	nulle ou très faible	fehlend oder sehr gering	ausente o muy bajo	Santhica 23	1
		medium	moyenne	mittel	medio	Usó 31	3
		very high	très élevée	sehr hoch	muy alto	Medisins	5

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
14.	2102	Plant: proportion of	Plante : proportion	Planze: Anteil	Planta: proporción	
(*)	2202	hermaphrodite	de plantes	zwittriger Pflanzen	de plantas	
(+)	2302	plants	hermaphrodites		hermafroditas	
	2304					
	MS/ VG					
QN	low	faible	gering	baja		1
	medium	moyenne	mittel	media		3
	high	élevée	hoch	alta		5
15.	2102	Plant: proportion of	Plante : proportion	Planze: Anteil	Planta: proporción	
(*)	2202	female plants	de plantes femelles	weiblicher Pflanzen	de plantas femeninas	
(+)	2302					
	2304					
	MS/ VG					
QN	low	faible	gering	baja		1
	medium	moyenne	mittel	media		3
	high	élevée	hoch	alta		5
16.	2102	Plant: proportion of	Plante : proportion	Planze: Anteil	Planta: proporción	
(*)	2202	male plants	de plantes mâles	männlicher Pflanzen	de plantas	
(+)	2302				masculinas	
	2304					
	MS/ VG					
QN	low	faible	gering	baja		1
	medium	moyenne	mittel	media		3
	high	élevée	hoch	alta		5
17.	2202	Plant: natural	Plante : hauteur	Pflanze: natürliche	Planta: altura	
(*)	2302	height	naturelle	Höhe	natural	
(+)	VG/ MG					
QN	short	basse	niedrig	baja	Finola	3
	medium	moyenne	mittel	media	Usó 31	5
	long	haute	hoch	alta	Ferimon	7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
18. 2202 (*) 2302 VG	Main stem: color	Tige principale : couleur	Haupttrieb: Farbe	Tallo principal: color		
PQ	(c) yellow	jaune	gelb	amarillo	Chamaeleon	1
	medium green	vert moyen	mittelgrün	verde medio	Epsilon 68	2
	dark green	vert foncé	dunkelgrün	verde oscuro	Kompolti	3
	purple	pourpre	purpurn	púrpura	Fibranova	4
19. 2202 2302 MS	Main stem: length of internode	Tige principale : longueur de l'entre-nœud	Haupttrieb: Internodienlänge	Tallo: longitud del entrenudo		
QN	(c) short	courte	niedrig	corto	Ferimon	3
	medium	moyenne	mittel	medio	Usó 31	5
	long	longue	lang	largo	KC Dora	7
20. 2202 2302 MS/ VG	Main stem: thickness	Tige principale : épaisseur	Haupttrieb: Dicke	Tallo principal: grosor		
QN	(c) thin	mince	dünn	delgado	Finola	1
	medium	moyenne	mittel	medio	Epsilon 68	2
	thick	épaisse	dick	grueso	Kompolti	3
21. 2202 2302 VG	Main stem: depth of grooves	Tige principale : profondeur des cannelures	Haupttrieb: Tiefe der Furchen	Tallo principal: profundidad de los surcos		
QN	(c) shallow	peu profondes	flach	poco profundos	Finola	1
	medium	moyennes	mittel	medios	Ferimon	2
	deep	profondes	tief	profundos	Dioica 88	3
22. 2204 2306 (+) VG	Main stem: pith in cross-section	Tige principale : moelle à la section transversale	Haupttrieb: Füllung im Querschnitt	Tallo principal: médula en sección transversal		
QN	(c) absent or thin	absente ou fine	fehlend oder dünn	ausente o muy delgada	Ermes	1
	medium	moyenne	mittel	media	Santhica 27	2
	thick	épaisse	dick	gruesa	Chamaeleon	3

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
23.	2205 Seed: 1,000 seed 2307 weight MG	Graine : poids de 1 000 graines	Samen: 1 000- Korngewicht	Semilla: peso de 1000 semillas		
QN	very low	très faible	sehr gering	muy bajo	Finola	1
	low	faible	gering	bajo	Chamaeleon	2
	medium	moyen	mittel	medio	Usó 31	3
	high	élevé	hoch	alto	Fedora 17	4
	very high	très élevé	sehr hoch	muy alto	Epsilon 68	5
24.	2205 Seed: color of testa 2307 VG	Graine : couleur des téguments	Samen: Farbe der Samenschale	Semilla: color del tegumento		
PQ	light grey	gris clair	hellgrau	gris ligero	Fibrol	1
	medium grey	gris moyen	mittelgrau	gris medio	Finola	2
	grey brown	brun gris	graubraun	marrón gris	Futura 75	3
	yellowish brown	brun jaunâtre	gelblichbraun	marrón amarillento	Santhica 27	4
	brown	brun	braun	marrón	Ermes	5
25.	2205 Seed: marbling 2307 (+) VG	Graine : marbrure	Samen: Marmorierung	Semilla: veteado		
QN	weak	faible	gering	débil	Finola	1
	medium	moyenne	mittel	medio	Kompolti	2
	strong	forte	stark	fuerte	Futura 75	3

8. Explanations on the Table of Characteristics

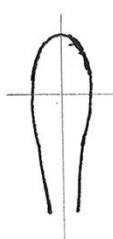
8.1 *Explanations covering several characteristics*

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

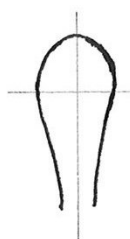
- (a) Observations should be done in the period between the beginning of flowering (growth stage 2101, 2201 or 2301, whichever is earliest) and the beginning of seed maturity.
- (b) Observations should be done on the last opposite, fully expanded leaves
- (c) Observations should be done on the internode below the last opposite leaves of female and/or hermaphrodite plants only.

8.2 *Explanations for individual characteristics*

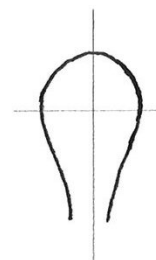
Ad. 1: Cotyledon: shape



1
narrow obovate



2
medium obovate



3
broad obovate

Ad. 8: Leaf: number of leaflets

Medium number of leaflets is 7 (predominant number of leaflets). Few is less than 7 leaflets. Many is more than 7 leaflets.

Ad. 11: Time of male flowering

Monoecious varieties: 50% of all plants with first staminate male flower open.

Other varieties: 50% of all male plants with first staminate male flower open.

First staminate flowers mostly appear from the axils of the leaves on the main stem. Staminate flowers usually appear about 2 weeks before the styles of pistillate female flowers are visible.

Ad. 13: Inflorescence: THC content

The method to determine the THC content is based on a quantitative determination of Δ^9 -tetrahydrocannabinol by gas chromatography after extraction with a suitable solvent.

Sampling

The sample (mixture of 20 plants) should be taken from the upper 30 cm of the main stem, containing the female inflorescence. Sampling should be carried out in the period from 20 days after the beginning of female flowering up to the end of flowering. The sample should be dried as soon as possible (within 48 hours) at a temperature below 60° C. Samples should be dried to a constant weight and to a moisture content of 8 – 13 %. After drying samples can be stored (without crushing) at below 25° C in a dark place.

Determination of THC content (see also Cole, 2003).

1. Preparation of the test sample

Remove stems and seeds over 2 mm in size from the dried samples.

Grind the dried samples to obtain a semi-fine powder (passing through a 1 mm mesh sieve).

The powder may be stored for 10 weeks at below 25° C in a dark dry place.

2. Reagents and extraction solution

Reagents

- Δ^9 -tetrahydrocannabinol, pure for chromatographic purposes.
- squalane, pure for chromatographic purposes, as an internal standard.

Extraction solution

- 35 mg of squalane per 100 ml hexane.

3. Extraction of Δ^9 -tetrahydrocannabinol

Weigh 100 mg of the powdered test sample, place in a centrifuge tube and add 5 ml of extraction solution containing the internal standard.

Place in an ultrasound bath and leave for 20 minutes. Centrifuge for 5 minutes at 3000 r.p.m. and then remove the supernatant THC solution. Inject the solution into the chromatograph and carry out a quantitative analysis.

4. Gas chromatography

(a) Apparatus

- gas chromatograph with a flame ionization detector and a split/splitless injector
- column allowing good separation of cannabinoids, for example a glass capillary column 25 m long and 0,22 mm in diameter impregnated with a 5% non-polar phenyl-methyl-siloxane phase.

(b) Calibration ranges

At least three points including points 0,04 and 0,50 mg/ml Δ^9 -THC in extraction solution.

(c) Experimental conditions

The following conditions are given as an example for the column referred to in a).

oven temperature	260° C
injector temperature	300° C
detector temperature	300° C

(d) Injection volume: 1 μ l

Results

THC should be determined to two decimals in grams of Δ^9 -THC per 100 grams of analytical sample dried to constant weight. A tolerance of 0,03 g per 100 grams applies. The results are expressed in % dry weight.

Although varietal differences for THC content are consistent, absolute levels of THC content are sensitive to environmental variation. States of expression need to be calibrated by Example varieties.

Ad. 14, 15 and 16: Plant: proportion of hermaphrodite plants, female plants and male plants resp.

Cannabis sativa L. is dioecious by nature, containing approximately equal proportions of male and female plants. Hermaphrodite plants (male and female flowers on one plant) occasionally occur naturally but are specially created by breeding activity (Bócsa, 1998). Several intersexual forms exist and sex expression can be modified by environmental factors.

Hermaphrodite plants: plants with both male and female flowers

Female plants: plants with female flowers only

Male plants: plants with male flowers only

Proportion	Note	Ranges (percentage)
low	1	=< 5 %
low to medium	2	6-35 %
medium	3	36-65 %
medium to high	4	66-95 %
high	5	> 95 %

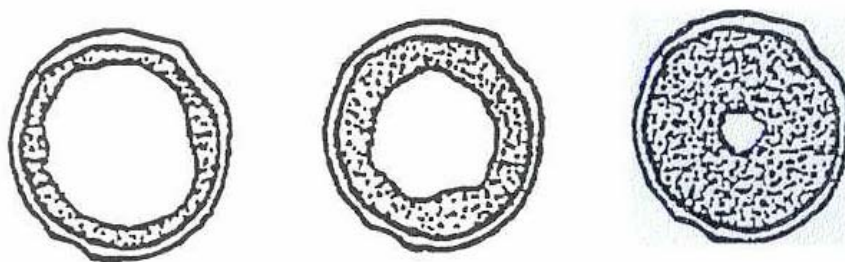
Proportion should be based on at least 200 plants for seed propagated varieties and at least 40 plants for vegetatively propagated varieties.

Ad. 17: Plant: natural height

Natural height should be observed on female and/or hermaphrodite plants including inflorescence.

Ad. 22: Main stem: pith in cross-section

Observations to be taken on internode below the last opposite leaves.



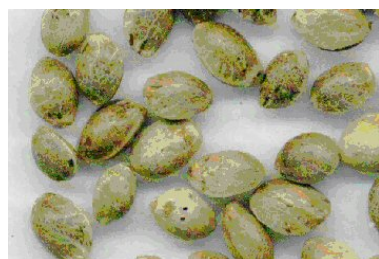
1
absent or thin

2
medium

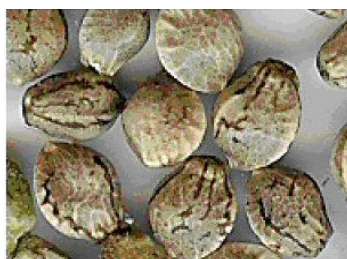
3
thick

Ad. 25: Seed: marbling

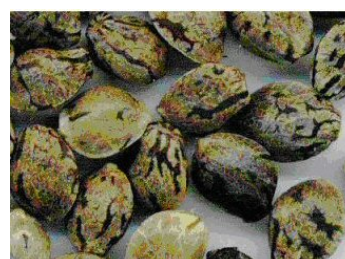
Marbling of testa: black mosaic patterns.



1
weak



2
medium



3
strong

8.3 *Growth stages for Hemp*

All characteristics should be recorded at the appropriate time for the plant concerned. Growth stages of hemp are recorded by a four-digit code describing the principal growth stages, depending on the sex of the plant followed by detailed developmental stages (Mediavilla, Vito *et al.*, 1998):

Principal growth stages

Four principal stages describe the life cycle of a plant and are coded by their first digit of the four-digit code.

First-digit of code	Definition
0	Germination and emergence
1	Vegetative stage
2	Flowering and seed formation
3	Senescence

Secondary growth stages

The secondary growth stages are described by the second digit, which indicates the sex of the plant, the third and fourth digits indicating the developmental stage of the plant.

Code	Definition	Remarks
Germination and emergence		
0000	Dry seed	
0003	Cotyledons unfolded	
Vegetative stage refers to main stem. Leaves are considered unfolded when leaflets are at least one cm long		
1002	1 st leaf pair	1 leaflet
1004	2 nd leaf pair	3 leaflets
1006	3 rd leaf pair	5 leaflets
10xx	Last opposite leaf pair	xx = 2 times n th leaf pair
Flowering and seed formation refers to the main stem including branches		
2000	GV point (i.e. induction of flowering)	Change of phyllotaxis on the main stem from opposite to alternate. Distance between petioles of alternate leaves at least 0.5 cm
2001	Flower primordia	Sex nearly indistinguishable
Male Plant		
2100	Flower formation	First closed staminate flowers
2101	Beginning of flowering	First opened staminate flowers
2102	Flowering	50% opened staminate flowers
2103	End of flowering	95% of staminate flowers opened or withered
Female Plant		
2200	Flower formation	First pistillate flowers Bract with no styles
2201	Beginning of flowering	Styles on first female flowers
2202	Flowering	50% of bracts formed
2203	Beginning of seed maturity	First seeds hard
2204	Seed maturity	50% of seeds hard
2205	End of seed maturity	95% of seeds hard or shattered
Hermaphrodite plant		
2300	Female flower formation	First pistillate flowers Perigonal bracts with no styles
2301	Beginning of female flowering	First styles visible
2302	Female flowering	50% of bracts formed
2303	Male flower formation	First closed staminate flowers
2304	Male flowering	50% opened staminate flowers
2305	Beginning of seed maturity	First seeds hard
2306	Seed maturity	50% of seeds hard
2307	End of seed maturity	95% of seeds hard or shattered
Senescence		
3001	Leaf dessication	Leaves dry
3002	Stem dessication	Leaves dropped
3003	Stem decomposition	Bast fibers free

9. Literature

Bócsa, I., 1998: Genetic Improvement : Conventional Approaches. In: Advances in Hemp Research. Paolo Ranalli (Ed.). Haworth Food Products Press, New York. 272 pp.

Bredemann, G., 1922 : Die Bestimmung des Fasergehaltes in Bastfaserpflanzen bei züchterischen Untersuchungen. Faserforschung 2. Leipzig : Hirzel Verlag. S. 239-258.

Clarke, R.C., 1998: Botany of the Genus *Cannabis*. In: Advances in Hemp Research. Paolo Ranalli (Ed.). Haworth Food Products Press, New York. 272 pp.

Cole, M.D., 2003. The analysis of controlled substances – a systematic approach. John Wiley and Sons Ltd., Chichester, UK. ISBN 0-471-49252-3.

Mediavilla, V., Jonquera, M., Schmid-Slembrouck, I., Soldati, A., 1998. Decimal code for growth stages of hemp (*Cannabis sativa* L.). Journal of the International Hemp Association 5(2) : 67-72.

Meijer de, E., 1995: Fibre hemp cultivars : A survey of origin, ancestry, availability and brief agronomic characteristics. Journal of the International Hemp Association 2(2) : 66-73

Meijer de, E., 1998: Cannabis Germplasm Resources. In: Advances in Hemp Research. Paolo Ranalli (Ed.). Haworth Food Products Press, New York. 272 pp.

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="Cannabis sativa L."/>	
1.2 Common name	<input type="text" value="Hemp"/>	
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"/>	
3. Proposed denomination and breeder's reference		
Proposed denomination (if available)	<input type="text"/>	
Breeder's reference	<input type="text"/>	

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

(.....) x (.....)
female parent male parent

(b) partially known cross []
(please state known parent variety(ies))

(.....) x (.....)
female parent male parent

(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

4.2.1 Seed-propagated varieties

- (a) Self-pollination
- (b) Cross-pollination
- (i) population
- (ii) synthetic variety
- (c) Other
(please provide details)

4.2.2 Vegetatively propagated varieties

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

- 4.2.3 Other
(please provide details)

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 Time of male flowering (11)		
very early	Finola	1[]
very early to early		2[]
early	Santhica 27	3[]
early to medium		4[]
medium	Dioica 88	5[]
medium to late		6[]
late	Futura 75	7[]
late to very late		8[]
very late	Kompolti	9[]
5.2 Inflorescence: THC content (13)		
absent or very low	Santhica 23	1[]
medium	Uso 31	3[]
very high	Medisins	5[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.3 Plant: proportion of hermaphrodite plants (14)		
low		1[]
low to medium		2[]
medium		3[]
medium to high		4[]
high		5[]
5.4 Plant: proportion of female plants (15)		
low		1[]
low to medium		2[]
medium		3[]
medium to high		4[]
high		5[]
5.5 Plant: proportion of male plants (16)		
low		1[]
low to medium		2[]
medium		3[]
medium to high		4[]
high		5[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.6 Plant: natural height (17)		
very short		1[]
very short to short		2[]
short	Finola	3[]
short to medium		4[]
medium	Uso 31	5[]
medium to long		6[]
long	Ferimon	7[]
long to very long		8[]
very long		9[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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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
<i>Example</i>	<i>Plant: natural height</i>	<i>short</i>	<i>long</i>
<p>Comments:</p>			

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

Main use

- | | | |
|-----|---------------------------|-----|
| (a) | bast fibre and woody core | [] |
| (b) | oil seed | [] |
| (c) | pharmaceuticals | [] |
| (d) | other | [] |
- (please provide details)

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 for where you have indicated "yes".

.....

9.3 Has the plant material to be examined been tested for the presence of virus or other pathogens?

Yes []

(please provide details as specified by the Authority)

No []

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature

Date

[End of document]