



PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

***Cannabis sativa* L.**

HEMP, CANNABIS

UPOV Code: CANNB_SAT

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CPVO-TP/276/2

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1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of *Cannabis sativa* L., including interspecific hybrids.

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (<http://www.upov.int/tgp/en/>) and the relevant UPOV Test Guideline TG/276/1 dated 28/03/2012 (<https://www.upov.int/edocs/tgdocs/en/tg276.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.02.2022**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report.

If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior pertinent agreement, on matters of particular urgency, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <https://public.plantvarieties.eu/publication> in the special issue S2/S3 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- 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.

2.3 Informing about problems on the submission of material

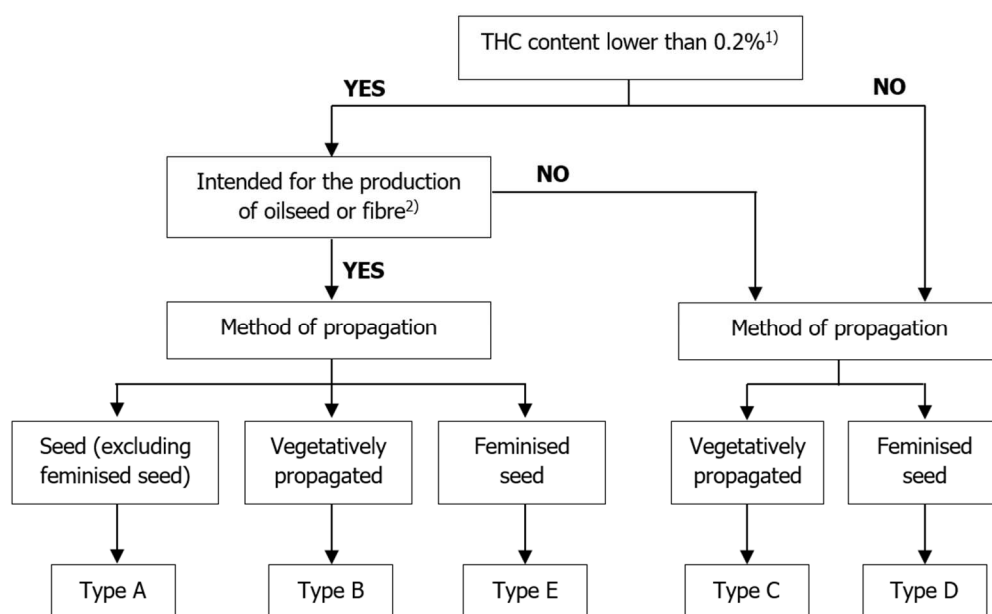
The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

3. METHOD OF EXAMINATION

As a first step, the collection should be divided according to the types as described in the Table below. In case of doubt to which type a variety belongs to, it should be tested under consideration of all relevant types.

Flow chart for classification into types:



¹⁾ Threshold for hemp/Cannabis cultivation according to EU regulation 1307/2013. The cultivation of varieties with a THC content higher than this threshold generally requires a permit. National (opium) laws may also apply.

²⁾ Varieties as referred to in EU Council Directive 2002/57/EC (13 June 2002) on the marketing of seed of oil and fibre plants.

3.1 Number of growing cycles

For types A, B and E, the minimum duration of tests should normally be two independent growing cycles.

For types C and D, the minimum duration of tests should normally be a single growing cycle.

The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

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" http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf.

3.3 Conditions for Conducting the Examination

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.

The optimum stage of development for the assessment of each characteristic is indicated by a number in the third column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.3

3.4 Test design

Varieties intended for the production of (oil)seed or fibre (types A, B and E) should be tested in the open field. Other varieties may be tested indoors.

For types A, and E, each test should be designed to result in a total of at least 200 plants, which should be divided between at least 2 replicates.

For type B, each test should be designed to result in a total of at least 60 plants, which should be divided between at least 2 replicates.

For type C, each test should be designed to result in a total of at least 10 plants.

For type D, each test should be designed to result in a total of at least 20 plants, which should be divided between at least 2 replicates.

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.

When tested indoors, special care should be taken to ensure normal growth and development, as most *Cannabis* varieties are highly sensitive to day length for flower induction and stress may affect sex expression.

3.5 Special tests for additional characteristics

In accordance with Article 23 of Implementing Rules N° 874/2009 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characteristics listed in the protocol.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge;

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties;

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection

(a) Types A, D and E

The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

(b) Types B and C

The variety collection shall comprise variety descriptions and may comprise living plant material. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material

(a) Types A, D and E

The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

(b) Types B and C

The EO shall obtain living plant material of reference varieties as and when those varieties need to be included in growing trials or other tests.

3.6.3 Range of the variety collection

The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO.

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection

The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue.

3.6.5 Maintenance and renewal/update of a living variety collection

(a) Types A, D and E

The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material, the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

(b) Types B and C

The EO shall maintain the variety collection under appropriate growing conditions (e.g., glasshouse, orchard, in vitro), where it shall be ensured that the plants are adequately irrigated, fertilised, pruned and protected from harmful pests and diseases. For the renewal of existing living material, the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material or by checking the identity of the new material against the variety description.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

4.1 Distinctness

4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

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 Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

If distinctness is assessed using the 2 x 1% criterion, the difference between two varieties is clear if the respective characteristics are significantly different in the same direction at the 1% level in at least two out of three years. The tests in each year are based on Student's two-tailed t-test of the differences between variety means with standard errors estimated using the residual mean square from the analysis of the variety x replicate plot means.

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p < 0.01$) in a test over either two or three years.

If the significance level or statistical methods proposed are not appropriate the method used should be clearly described.

4.1.4 Number of plants/parts of plants to be examined

For types A, B and E, 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.

For type C, unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 5 plants or parts taken from each of 5 plants and any other observations made on all plants in the test, disregarding any off-type plants.

For type D, unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts taken from each of 10 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 third 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. colour 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 this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

4.2.2 This Technical Protocol has been developed for the examination of cross-pollinated varieties (type A), vegetatively propagated varieties (types B and C), and varieties propagated by feminised seed (type D). For varieties with other types of propagation the recommendations in the UPOV-General Introduction to DUS and document TGP/13 "Guidance for new types and species", Section 4.5 "Testing Uniformity" should be followed.

Type A:

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

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 1% significance level or less ($p < 0.01$) in a test over two consecutive cycles.

If uniformity is assessed by the relative variance method the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the threshold limit for relative variance, taking into account the sample size, in a test over either two or three consecutive cycles (e.g. for a sample size of 20 measurements the threshold limit ($P = 0.01$) for relative variance is 1.88).

If uniformity is assessed by off-types, a population standard of 3% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 200 plants, 10 off-types are allowed.

Type B:

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 60 plants, 2 off-types are allowed.

Type E:

For the assessment of uniformity of varieties propagated by feminised seed, a population standard of 2% and an acceptance probability of at least 95 % should be applied. In the case of a sample size of 200 plants, 7 off-types are allowed.

If uniformity is assessed by the relative variance method the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the threshold limit for relative variance, taking into account the sample size, in a test over either two or three consecutive cycles (e.g. for a sample size of 20 measurements the threshold limit ($P = 0.01$) for relative variance is 1.88).

Type C:

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 10 plants, 1 off-type is allowed.

Type D:

For the assessment of uniformity of varieties propagated by feminised seed, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 20 plants, 2 off-types are allowed.

If uniformity is assessed by the relative variance method the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the threshold limit for relative variance, taking into account the sample size, in a test over either two or three consecutive cycles (e.g. for a sample size of 10 measurements the threshold limit ($P = 0.01$) for relative variance is 2.32).

4.3 Stability

- 4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf)

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 ORGANISATION 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 organise the growing trial so that similar varieties are grouped together.
- 5.3** The following have been agreed as useful grouping characteristics:
- a) Number of leaflets (characteristic 5)
 - b) Central leaflet: width (characteristic 7)
 - c) Only varieties of types A: Time of male flowering (characteristic 8)
 - d) Only varieties of types B, C, D and E: Time of female flowering (characteristic 9)
 - e) Plant: proportion of hermaphrodite plants (characteristic 12)
 - f) Plant: proportion of female plants (characteristic 13)
 - g) Plant: proportion of male plants (characteristic 14)
 - h) Only varieties of types A, B and E: Plant: natural height (characteristic 18)
 - i) Only varieties of types C and D: Plant: height (characteristic 19)
 - j) Main stem: colour (characteristic 20)
 - k) Only varieties of types A, B and E: Inflorescence: THC content (characteristic 26)
 - l) Only varieties of types C and D: Inflorescence: THC content (characteristic 27)
 - m) Inflorescence: CBD content (characteristic 28)
- 5.4** If other characteristics than those from the Technical Protocol are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.
- 5.5** Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted or by specific legislation on plant health. In the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

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. All relevant states of expression are presented in the characteristic.

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

6.3 Example Varieties

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

6.4 Legend

For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2

For column 'UPOV N°':

The numbering of the characteristics is provided as a reference to the UPOV guideline.

(*) UPOV Asterisked characteristic: Characteristics that are important for the international harmonization of variety descriptions.

For column 'Stage, method':

MG, MS, VG, VS -see Chapter 4.1.5

(a)-(c) Explanations covering several Characteristics -see Chapter 8.1

0000-3003 Explanations on growth stages -see Chapter 8.3

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1. QL	1.	VG (a)	Leaf: variegation absent	Futura 75 (type A), Aida (type C)	1
			present	Divina (type C)	9
2. QN	5.	VG (a)	Only varieties with leaf variegation: absent: Leaf: intensity of green colour light	Fibror 79 (type A), Aida (type C)	1
			medium	Fedora 17 (type A), Theresa (type C)	2
			dark	Finola (type A), Gill (type C)	3
3. QN	6.	MS/VG (a)	Leaf: length of petiole short	Fibrol (type A), MGC 1013 (type C)	1
			(b) medium	Fedora 17 (type A), Bedrolite (type C), Divina (type C)	2
			long	Carmagnola (type A)	3
4. QN	7. (*)	VG (a)	Leaf: anthocyanin coloration of petiole absent or very weak	Fibrol (type A), Gill (type C)	1
			(b) weak	Ruby (type A), Theresa (type C)	2
			medium	Dioica 88 (type A), Gayle (type C)	3
			strong	M-1337 (type C)	4
			very strong	Finola (type A), EVLS 113 (type C)	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note		
5. (+)	8. (*)	MS/VG	Leaf: number of leaflets				
			QN	(a)	very few	Bedrolite (type C), MGC 1013 (type C)	1
				(b)	few	Finola (type A), Aida (type C)	2
					medium	USO 31 (type A), GRX53 (type D)	3
					many	Fibror 79 (type A)	4
G			very many		5		
6. QN	9.	MS/VG	Central leaflet: length				
			(a)	very short	Damato Red (type C)	1	
			(b)	very short to short	MGC 1013 (type C)	2	
				short	Divina (type C)	3	
				short to medium		4	
				medium	Aida (type C)	5	
				medium to long		6	
				long	Felina 32 (type A)	7	
				long to very long		8	
	very long	Carmagnola (type A)	9				
7. QN	10.	MS/VG	Central leaflet: width				
			(a)	very narrow		1	
			(b)	very narrow to narrow	Celeste (type C)	2	
				narrow	MGC 1013 (type C)	3	
				narrow to medium		4	
				medium	Fibrol (type A), Theresa (type C)	5	
				medium to broad	Hulkberry (type C)	6	
				broad	USO 31 (type A), Gill (type C)	7	
				broad to very broad		8	
G			very broad	Carmagnola (type A), Enectabis (type D)	9		

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
8. (+)	11. (*)	MG/VG	Only varieties of type A: Time of male flowering		
QN			very early	USO 31	1
			very early to early		2
			early		3
			early to medium		4
			medium	Fibrol	5
			medium to late		6
			late	Felina 32	7
			late to very late		8
G			very late	Dioica 88	9
9. (+)		MG/VG	Only varieties of types B, C, D and E: Time of female flowering		
QN			very early	Celeste (type C)	1
			very early to early		2
			early	Theresa (type C)	3
			early to medium		4
			medium	M-1337 (type C)	5
			medium to late		6
			late	Goya (type C)	7
			late to very late		8
G			very late	HURV2019PL (type D)	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
10.	12.	2102 2304	Only varieties of types A: Inflorescence: anthocyanin coloration of male flowers		
QN		VG	absent or very weak	Santhica 27	1
			very weak to weak		2
			weak	USO 31	3
			weak to medium		4
			medium	Felina 32	5
			medium to strong		6
			strong	Adzelviesi	7
			strong to very strong	Finola	8
			very strong		9
11. (+)		2202b 2302b	Only varieties of types B, C, D and E: Inflorescence: anthocyanin coloration of female flowers		
QN		VG	absent or weak	Aida (type C)	1
			medium		2
			strong	HURV2019PL (type D)	3
12. (+)	14. (*)	2102 2202 2302 2304	Plant: proportion of hermaphrodite plants		
QN		MS/VG	low		1
			low to medium		2
			medium		3
			medium to high		4
G			high		5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note		
13. (+)	15. (*)	2102 2202 2302 2304	Plant: proportion of female plants				
			QN	MS/VG	low		1
					low to medium		2
					medium		3
					medium to high		4
G		high		5			
14. (+)	16. (*)	2102 2202 2302 2304	Plant: proportion of male plants				
			QN	MS/VG	low		1
					low to medium		2
					medium		3
					medium to high		4
G		high		5			
15. (+)		2202b 2302b	Only varieties of types C and D:				
			Flower: length of stigmas				
			QN	VG	short	EVLS 113 (type C)	1
medium	Divina (type C)	2					
long	Bedrobinol (type C), HURV2019PL (type D)	3					
16.		2202b 2302b	Only varieties of types C and D:				
			Flower: thickness of stigmas				
			QN	VG	thin	HURV2019CBG (type C)	1
medium	Divina (type C)	2					
thick	HURV2019PL (type D)	3					

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
17. (+)		2202b 2302b	Only varieties of types C and D: Flower: contortion of stigmas		
QN		VG	absent or weak	Aida (type C)	1
			medium	MGC1008 (type C), HURV2019PL (type D)	2
			strong	MGC1009 (type C)	3
18. (+)	17. (*)	2202 2302	Only varieties of types A, B and E: Plant: natural height		
QN		MG/VG	very short	Finola (type A), Adzelviesi (type A)	1
			very short to short		2
			short		3
			short to medium		4
			medium	Usó 31 (type A)	5
			medium to long	Fibrol (type A)	6
			long	Felina 32 (type A)	7
			long to very long	Fibror 79 (type A)	8
G			very long	Dioica 88 (type A)	9
19. (+)	17.	2202b 2302b	Only varieties of types C and D: Plant: height		
QN		MG/VG	very short	MGC 1027 (type C)	1
			very short to short		2
			short	Chuy (type C)	3
			short to medium		4
			medium	Aida (type C)	5
			medium to long		6
			long	Bedrolite (type C), EVLS 113 (type C)	7
			long to very long	Obi (type D)	8
G			very long		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
20.	18. (*)	2202 2202b 2302 2302b	Main stem: colour		
PQ		VG	yellow	Fibror 79 (type A)	1
		(c)	medium green	Felina 32 (type A), Bedrobinol (type C), Theresa (type C)	2
			dark green	Dioica 88 (type A), Aida (type C)	3
G			purple	Fibranova (type A), EVLS 113 (type C)	4
21.	19.	2202 2202b 2302 2302b	Only varieties of types A, B and E: Main stem: length of internode		
QN		MS/VG	very short	Finola (type A)	1
		(c)	very short to short		2
			short		3
			short to medium		4
			medium	Uso 31 (type A)	5
			medium to long		6
			long	Futura 75 (type A)	7
			long to very long		8
			very long		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
22.		2202 2202b 2302 2302b	Only varieties of types C and D: Main stem: length of internode		
QN		MS/VG	very short		1
		(c)	very short to short	MGC 1027 (type C)	2
			short	Beatriz (type C), Divina (type C)	3
			short to medium		4
			medium	Aida (type C), HURV2019PL (type D)	5
			medium to long	EVLS 113 (type C)	6
			long		7
			long to very long		8
			very long	Enectitaca (type D), Obi (type D)	9
23.	20.	2202 2302	Only varieties of types A, B and E: Main stem: thickness		
QN		MS/VG	thin	Finola (type A)	1
		(c)	medium	Futura 75 (type A)	2
			thick	Dioica 88 (type A)	3
24.	20.	2202b 2302b	Only varieties of types C and D: Main stem: thickness		
QN		MS/VG	thin	Celeste (type C)	1
		(c)	medium	Aida (type C)	2
			thick	Obi (type D)	3
25.	21.	2202 2202b 2302 2302b	Main stem: depth of grooves		
QN		VG	shallow	Finola (type A), Divina (type C), Gill (type C)	1
		(c)	medium	Fedora 17 (type A), Bedrolite (type C), Theresa (type C)	2
			deep	Dioica 88 (type A), HURV2019PL (type D)	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
26. (+)	13. (*)	2204 2204b 2305 2305b	Only varieties of types A, B and E: Inflorescence: THC content		
QN		MG	absent or very low	Santhica 27 (type A)	1
			very low to low	Fedora 17 (type A)	2
G			low	Futura 75 (type A)	3
27. (+)	13. (*)	2204 2204b 2305 2305b	Only varieties of types C and D: Inflorescence: THC content		
QN		MG	absent or very low	Aida (type C)	1
			very low to low	Bedrolite (type C), Sara (type C)	2
			low		3
			low to medium	Beatriz (type C)	4
			medium	Bediol (type C)	5
			medium to high	Bedrobinol (type C), Raquel (type C)	6
			high	Bedrocan (type C), GRX53 (type D)	7
			high to very high	Original Blitz (type C)	8
G			very high		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
28. (+)		2204 2204b 2305 2305b	Inflorescence: CBD content		
QN		MG	absent or very low	Santhica 27 (type A), Bedrobinol (type C), Bedrocan (type C), Raquel (type C), Enectacalm (type D)	1
			very low to low	Fedora 17 (type A)	2
			low	Futura 75 (type A), Theresa (type C), Aida (type C)	3
			low to medium	Chuy (type C)	4
			medium	Bediol (type C), Divina (type C)	5
			medium to high	Sibari (type C)	6
			high	Goya (type C)	7
			high to very high	A1 Philadelphia (type C), Enectonica (type D)	8
G			very high		9
29. (+)	22.	2204 2204b 2306 2306	Main stem: pith in cross-section		
QN		VG	absent or thin	Santhica 27 (type A), HURV2019PL (type D)	1
		(c)	medium	Fedora 17 (type A), Divina (type C)	2
			thick	Finola (type A), Gill (type C), MGC1009 (type C)	3
30.	23.	2205 2307	Seed: 1,000 seed weight		
QN		MG	very low	Finola (type A)	1
			low	Chamaeleon (type A), Enectitaca (type D)	2
			medium	Felina 32 (type A), Enectacalm (type D)	3
			high	Santhica 27 (type A)	4
			very high	Fibror 79 (type A)	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
31.	24.	2205 2307	Seed: colour of testa		
PQ		VG	light grey	Finola (type A)	1
			medium grey	USO 31 (type A), Enectavio (type D)	2
			grey brown	Fedora 17 (type A), Enectacalm (type D)	3
			yellowish brown	Fibror 79 (type A)	4
			brown	Dioica 88 (type A), Enectitaca (type D)	5
32. (+)	25.	2205 2307	Seed: marbling		
QN		VG	weak	Finola (type A), Enetacalm (type D)	1
			medium	Felina 32 (type A), Enectavio (type D)	2
			strong	Dioica 88 (type A)	3

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

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

- (a)¹ 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 (Type A) or flower senescence (Types B, C, D and E).
- (b)² For type A, observations should be done on the last opposite, fully expanded leaves. For Types B, C, D and E observations should be done on fully developed leaves from the centre of the plant.
- (c)² For type A, observations should be done on the internode below the last opposite leaves of female and/or hermaphrodite plants only. In case of types B, C, D and E, observations should be done on the internode below a fully developed leaf from the centre of the plant.

¹In the absence of male plants/flowers, female plants/flowers generally do not set seed. Under those circumstances, plant maturity should be assessed according to flower senescence.

²Vegetatively propagated and feminised seed plants generally do not show a GV point (the place on the stem where the phyllotaxis changes from opposite to alternate).

8.2 Explanations for individual characteristics

Ad. 5: Leaf: number of leaflets

The predominant number of leaflets in the centre of the plant should be observed:

- 1 - very few = three leaflets or less
- 2 - few = five leaflets
- 3 - medium = seven leaflets
- 4 - many = nine leaflets
- 5 - very many = eleven leaflets or more

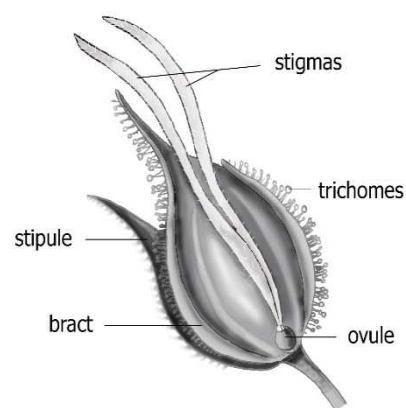
Ad. 8-17: Flowering characteristics



Male flower



Female flower



Longitudinal cross-section of female flower

Ad. 8: Only varieties of types A: Time of male flowering

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

First male flowers mostly appear from the axils of the leaves on the main stem. Male flowers usually appear about 2 weeks before the stigmas of female flowers are visible.

Ad. 9: Only varieties of types B, C, D and E: Time of female flowering

Vegetatively propagated and feminised seed varieties: 50% of plants with first stigmas visible

Ad. 11: Only varieties of type B, C, D and E: Inflorescence: anthocyanin coloration of female flowers.

The colour of the bracts, stipules and sugarleaves³ should be observed.

³) Sugarleaves are the leaves between the clusters of female flowers.



1
absent or weak



3
Strong

Ad. 12, 13 and 14: Plant: proportion of hermaphrodite plants, female plants and male plants resp.

Type A:

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	>= 96 %

Proportion should be based on at least 200 plants.

Types B and C:

Vegetatively propagated varieties should show only one type of sex expression.

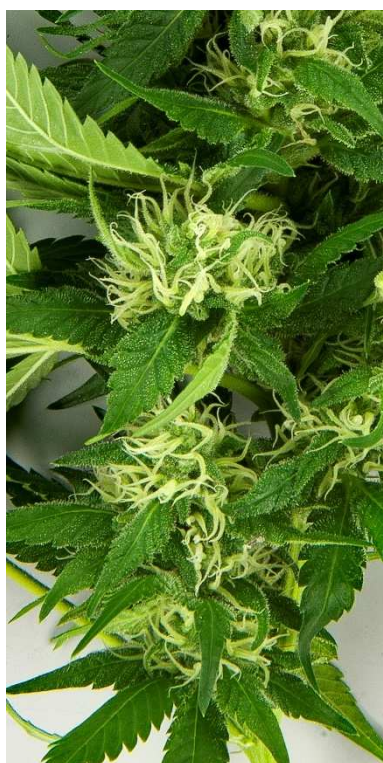
Types D and E:

Feminised seed varieties may be female, hermaphrodite or may show a mixture of female and hermaphrodite plants. Sex expression is affected by environmental conditions and stress.

Ad. 17: Only varieties of types C and D: Flower: contortion of stigmas



1
absent or weak



2
Medium



3
strong

Ad. 18 and 19: Plant height

Plant height should be observed on female and/or hermaphrodite plants from soil level to top of the plant including inflorescence.

Ad. 26, 27 and 28: Inflorescence: THC and CBD content

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

Sampling

The sample should be taken from the upper 30 cm of the main stem, containing well developed female inflorescences.

Types A, B and E: a mixture of 20 plants

Type C: a mixture of 5 plants

Type D: a mixture of 10 plants

(Sugar-)leaves should be removed as much as possible.

The sample should be dried as soon as possible (within 48 hours) at a temperature below 70° 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/CBD content (Adapted from: Commission Delegated Regulation (EU) No 639/2014 annex II (latest amended version)).

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 (THC), pure for chromatographic purposes.
- Cannabidiol (CBD), 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 cannabinoids

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 3,000 r.p.m. and then remove the supernatant cannabinoid 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 of each of the cannabinoids 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 and CBD should be determined to two decimals in grams of Δ^9 -THC and CBD resp., per 100 grams of analytical sample dried to constant weight. A tolerance of 0.03 g per 100 grams applies.

Although varietal differences for THC and CBD content are consistent, absolute levels of THC and CBD content are sensitive to environmental variation.

For listing purposes (EU regulation 1307/2013), the THC content of variety types A, B and E should not exceed 0.2%.

Ad. 29: Main stem: pith in cross-section



1
absent or thin



2
medium



3
Thick

Ad. 32: Seed: marbling

Marbling of testa: black mosaic patterns



1
weak



2
medium



3
strong

8.3 Explanations on growth stages

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). This growth scale is slightly modified by adding definitions of certain stages (marked by *), to accommodate types B, C, D and E when no seed is formed. Seed formation affects the production of cannabinoids as THC and CBD and is therefore avoided for types C and D. Stages with the same number indicate the same growth stage (e.g. 1006=1006b).

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
1006b*	5 th leaf	In vegetatively propagated plants all leaves have the same phyllotaxis
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. In vegetatively propagated plants the GV point is absent, all leaves have the same phyllotaxis.
2001	Flower primordia	Sex nearly distinguishable
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 stigmas
2201	Beginning of flowering	Stigmas on first female flowers
2202	Flowering	50 % of bracts formed
2202b*	Flowering	50% of stigmas fully extended
2203	Beginning of seed maturity	First seeds hard
2203b*	Flowers senescence	10% of stigmas brown
2204	Seed maturity	50 % of seeds hard
2204b*	End of flowering	50% of stigmas brown
2205	End of seed maturity	95 % of seeds hard or shattered
Hermaphrodite plant		
2300	Female flower formation	First pistillate flowers. Perigonal bracts with no stigmas
2301	Beginning of female flowering	First stigmas visible

2302	Female flowering	50 % of bracts formed
2302b*	Female flowering	50% of stigmas fully extended
2303	Male flower formation	First closed staminate flowers
2304	Male flowering	50 % opened staminate flowers
2305	Beginning of seed maturity	First seeds hard
2305b*	Female flower senescence	10% of stigmas brown
2306	Seed maturity	50 % of seeds hard
2306b*	End of female flowering	50% of stigmas brown
2307	End of seed maturity	95 % of seeds hard or shattered
	Senescence	
3001	Leaf desiccation	Leaves dry
3002	Stem desiccation	Leaves dropped
3003	Stem decomposition	Bast fibres free

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the [CPVO website](#) under the following reference:
CPVO-TQ/276/2 – *Cannabis sativa* L. – hemp, cannabis

Link to e-TQ:

<https://applyfor.plantvarieties.eu/myprv.oa/#!/en/oa/show/questionnaire/TQ/13137/en>