



CPVO · OCVV

Community Plant Variety Office
Office Communautaire des Variétés Végétales

CPVO/TP-276/1-Partial Rev
Date: 21/03/2018

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

***Cannabis sativa* L.**

HEMP

UPOV Species Code: CANNB_SAT

Adopted on 21/03/2018

Entered into force on 21/03/2018

I SUBJECT OF THE PROTOCOL

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 general UPOV Document TG/1/3 and UPOV Guideline TG/276/1 dated 28/03/2012 (<http://www.upov.int/edocs/tgdocs/en/tg276.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of *Cannabis sativa* L..

II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the Examination Office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection of the Examination Office as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO if no plant material has been received. However, if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Seed requirements

The final dates for request for technical examination and sending of Technical Questionnaire as well as the submission date of plant material by the applicant, and quantity and quality of the plant material to be supplied by the applicant are published on the CPVO web site (<http://cpvo.europa.eu/applications-and-examinations/technical-examinations/submission-of-plant-material-s2-publication>) in S2 Official Gazette.

Quality of seed/plant material: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.

Seed Treatment:The plant material must not have undergone any treatment unless the CPVO and the examination office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Labelling of sample:- Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination Office reference (if known)
- Name of applicant
- The phrase "On request of the CPVO".

III CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation (EC) No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.
- In case of hybrids, all components of hybrid varieties in common knowledge must be considered as part of the reference collection.

The composition of the variety collection in each Examination Office depends on the ecological conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other offices involved in DUS-testing of hemp. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. 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. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation (EC) No. 874/2009, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics that could be used for grouping are the following (CPVO numbering; G for grouping in table of characteristics)

- a) Time of male flowering (characteristic 8)
- b) Inflorescence: THC content (characteristic 10)
- c) Plant: proportion of hermaphrodite plants (characteristic 11)
- d) Plant: proportion of female plants (characteristic 12)
- e) Plant: proportion of male plants(characteristic 13)
- f) Plant: natural height (characteristic 14)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles for seed propagated varieties and one growing cycle for vegetatively propagated varieties. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing cycle.

The test design is as follows:

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.

In the case of vegetatively propagated varieties, each test should be designed to result in a total of at least 20 plants.

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.

Unless otherwise indicated, for the purpose 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.

6. Special tests

In accordance with Article 83(3) of Council Regulation (EC) No. 2100/94 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 characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation (EC) No. 2100/94.

Qualitative characteristics:

In the case of characteristics which show discrete discontinuous states of expression, a difference between two varieties is clear if the respective characteristics have expressions which fall into two different states.

Quantitative characteristics:

Characteristics which show a continuous range of expression from one extreme to the other may be either measured or visually observed.

In the case of characteristics assessed by a single observation of a group of plants or parts of plants (VG, MG), a difference between two varieties is clear if the expression of the respective characteristics differs by at least the span of one note, taking into account the variability observed within the varieties.

For two varieties to be distinct using the 2 x 1% criterion, the varieties need to be significantly different in the same direction at the 1% level in at least two out of three years in one or more measured characteristics. 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 the statistical methods proposed are not appropriate the method used should be clearly described.

b) **Uniformity**

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

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.

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 20 plants, 1 off-type is allowed.

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity. Seed samples of further submissions included in any test must show the same expression of characteristics as the material originally supplied.

IV - REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing cycles but in some cases three growing cycles may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the Examination Office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V - LIAISON WITH THE APPLICANT

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

VI - ENTRY INTO FORCE

The present protocol enters into force on **21.03.2018**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the new TP. 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 the submission of plant material for the first growing period.

In cases where the CPVO requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process of being carried out at the moment of the 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.

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 <u>Legend:</u>	
(+)	See explanations on the Table of characteristics
G	Grouping characteristic
 <u>Types of expression of characteristics:</u>	
QL	Qualitative characteristic
QN	Quantitative characteristic
PQ	Pseudo-qualitative characteristic
 <u>Type of observation of characteristics:</u>	
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
 <u>Type of observation: visual (V) or measurement (M)</u>	
"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.	
 <u>Type of record: for a group of plants (G) or for single, individual plants (S)</u>	
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.	
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ANNEX I

TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples ²	Note
1. QN	4.	1006	Plant: anthocyanin coloration of crown		
		VG	absent or very weak		1
			weak	Felina 32	3
			medium	Epsilon 68	5
			strong	Finola	7
2. QN	5.	VG	Leaf: intensity of green color		
		(a)	light	Chamaeleon	1
			medium	Fedora 17	2
		dark	Epsilon 68	3	
3. QN	6.	MS	Leaf: length of petiole		
		(a)	short	Santhica 27	1
		(b)	medium	Fedora 17	2
		long	Ermes	3	
4. QN	7.	VG	Leaf: anthocyanin coloration of petiole		
		(a)	absent or very weak	Fibrol	1
		(b)	weak	Ruby	2
			medium	Dioica 88	3
			strong	Epsilon 68	4
		very strong	Finola	5	
5. (+) QN	8.	MS/VG	Leaf: number of leaflets		
		(a)	few	Ermes	1
		(b)	medium	Epsilon 68	2
		many	Kompolti	3	

¹ The optimum stage of development as well as method of observation for the assessment of each characteristic is indicated by numbers and letters. Explanations are given in Annex 1 in 'Explanations and Methods'.

² Example varieties, separated by a semicolon, are indicated for winter barley before the semicolon, for spring barley they follow the semicolon. Example varieties are given as an indication, others may be used.

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples2	Note
6. QN	9.	MS (a) (b)	Central leaflet: length		
			short	Santhica 27	3
			medium	Epsilon 68	5
			long	Kompolti	7
7. QN	10.	MS (a) (b)	Central leaflet: width		
			narrow	Santhica 27	3
			medium	Dioica 88	5
			broad	Kompolti	7
8. (+) QN G	11.	MG	Time of male flowering		
			very early	Finola	1
			early	Santhica 27	3
			medium	Dioica 88	5
			late	Futura 75	7
			very late	Kompolti	9
9. QN	12.	2102 2304 VG	Inflorescence: anthocyanin coloration of male flowers		
			absent or very weak	Kompolti	1
			weak	Beniko	3
			medium	Uso 31	5
			strong	Ermes	7
			very strong	Finola	9
10. (+) QN G	13.	MG	Inflorescence: THC content		
			absent or very low	Santhica 23	1
			medium	Uso 31	3
			very high	Medisins	5
11. (+) QN G	14.	2102 2202 2302 2304 MS/VG	Plant: proportion of hermaphrodite plants		
			low		1
			medium		3
			high		5

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples2	Note		
12.	15.	2102 2202 2302 2304	Plant: proportion of female plants				
			(+)	MS/VG	low	1	
			QN		medium	3	
			G		high	5	
13.	16.	2102 2202 2302 2304	Plant: proportion of male plants				
			(+)	MS/VG	low	1	
			QN		medium	3	
			G		high	5	
14.	17.	2202 2302	Plant: natural height				
			(+)	VG/M G	short	Finola	3
			QN		medium	Usó 31	5
			G		long	Ferimon	7
15.	18.	2202 2302	Main stem: color				
			PQ	VG	yellow	Chamaeleon	1
				(c)	medium green	Epsilon 68	2
					dark green	Kompolti	3
					purple	Fibranova	4
16.	19.	2202 2302	Main stem: length of internode				
			QN	MS	short	Ferimon	3
				(c)	medium	Usó 31	5
					long	KC Dora	7

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples2	Note
17.	20.	2202 2302	Main stem: thickness		
QN		MS/VG	thin	Finola	1
		(c)	medium	Epsilon 68	2
			thick	Kompolti	3
18.	21.	2202 2302	Main stem: depth of grooves		
QN		VG	shallow	Finola	1
		(c)	medium	Ferimon	2
			deep	Dioica 88	3
19.	22.	2204 2306	Main stem: pith in cross-section		
(+)		VG	absent or thin	Ermes	1
QN		(c)	medium	Santhica 27	2
			thick	Chamaeleon	3
20.	23.	2205 2307	Seed: 1,000 seed weight		
QN		MG	very low	Finola	1
			low	Chamaeleon	2
			medium	Uso 31	3
			high	Fedora 17	4
			very high	Epsilon 68	5
21.	24.	2205 2307	Seed: color of testa		
PQ		VG	light grey	Fibrol	1
			medium grey	Finola	2
			grey brown	Futura 75	3
			yellowish brown	Santhica 27	4
			brown	Ermes	5

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples2	Note
22.	25.	2205 2307	Seed: marbling		
(+)		VG	weak	Finola	1
QN			medium	Kompolti	2
			strong	Futura 75	3

EXPLANATIONS AND METHODS

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.

Explanations for individual characteristics

Ad. 5: Leaf: number of leaflets

Few is less than 7 leaflets.

Medium number of leaflets is 7 (predominant number of leaflets).

Many is more than 7 leaflets.

Ad. 8: 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 styles of female flowers are visible.

Ad. 10: 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 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 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 3,000 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. 11, 12 and 13: 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	>= 96 %

Proportion should be based on at least 200 plants for seed propagated varieties and at least 40 plants for vegetatively propagated varieties (numbers are rounded to whole numbers).

Ad. 14: Plant: natural height

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

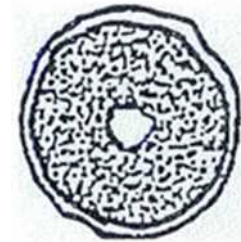
Ad. 19: Main stem: pith in cross-section



1
absent or thin



2
medium



3
thick

Ad. 22: Seed: marbling

Marbling of testa: black mosaic patterns



1
weak



2
medium



3
strong

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

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/276/1