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## INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

## DRAFT

## HEMP, CANNABIS

UPOV Code(s): CANNB_SAT
Cannabis sativa L .

## GUIDELINES

## FOR THE CONDUCT OF TESTS

## FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from the Netherlands to be considered by the
Technical Working Party for Agricultural Crops at its fifty-second session, to be held virtually

> from 2023-05-22 to 2023-05-26

Disclaimer: this document does not represent UPOV policies or guidance
Alternative names:*

| Botanical name | English | French | German | Spanish |
| :--- | :--- | :--- | :--- | :--- |
| Cannabis sativa L. | Cannabis, Hemp | Chanvre, Cannabis | 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.
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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, feminized seed, or rooted cuttings.
2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

Type A (fibre and seed production): 500 gr seeds
Type B (fibre and seed production): 60 rooted cuttings Type E (fibre and seed production): 500 gr of feminized seeds Type C (uses other than fibre or seed production): 15 rooted cuttings Type D (uses other than fibre or seed production): 500 feminized seeds


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
3.1.1 The minimum duration of tests should normally be two independent growing cycles.
3.1.2 The two independent growing cycles should be in the form of two separate plantings.
3.1.3 For types C and D , the minimum duration of tests should normally be a single growing cycle when tests are performed in a controlled environment.
3.1.4 In case of doubt to which type a variety belongs, it should be tested under consideration of all relevant types.
3.1.5 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".
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 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 and feminized seed propagated varieties (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.
3.4.2 In the case of vegetatively propagated varieties (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.
3.4.3 In the case of vegetatively propagated varieties (type C), each test should be designed to result in a total of at least 10 plants.
3.4.4 In the case of feminized seed propagated varieties (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.
3.4.5 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 or Parts of Plants to be Examined

In the case of seed-propagated varieties (type A), 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 observation made on all plants in the test, disregarding any off-type plants.

In the case of vegetatively propagated varieties (type B), 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 observation made on all plants in the test, disregarding any off-type plants.

In the case of vegetatively propagated varieties (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 observation made on all plants in the test, disregarding any off-type plants.

In the case of feminized seed-propagated varieties (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 observation made on all plants in the test, disregarding any off-type plants.

In the case of feminized seed-propagated varieties (type 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 observation 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 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 $(\mathrm{V})$ 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 $(\mathrm{G})$, 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 These Test Guidelines have been developed for the examination of cross-pollinated (type A), vegetatively propagated (types B and C), and feminized seed propagated (types $D$ and $E$ ) varieties. For varieties with other types of propagation, the recommendations in the General Introduction and document TGP/13 "Guidance for new types and species" Section 4.5 "Testing Uniformity" should be followed.
4.2.3 The assessment of uniformity for type A varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.
4.2.4 In type A varieties, for the characteristics Leaf: variegation and Main stem: color, 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.
4.2.5 For the assessment of uniformity of vegetatively propagated varieties (type B), 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.

For the assessment of vegetatively propagated varieties (type C), 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.

For the assessment of feminized seed propagated varieties (type D), 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.

For the assessment of feminized seed propagated varieties (type E), 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.

### 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.
5.3 The following have been agreed as useful grouping characteristics:
(a) Leaf: number of leaflets (characteristic 5)
(b) Central leaflet: width (characteristic 7)
(c) Only varieties of type 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 monoecious 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: color (characteristic 20)
(k) Only varieties of types A, B and E: Inflorescence: THC content (characteristic 26)
(I) Only varieties of types C and D: Inflorescence: THC content (characteristic 27)
(m) Inflorescence: CBD content (characteristic 28)
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 All relevant states of expression are presented in the characteristic.
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 pseudoqualitative) 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.

Legend

|  |  | English |  | français |  | deutsch | español | Example Varieties <br> Exemples <br> Beispielssorten <br> Variedades ejemplo | Note/ Nota |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |  |  |  |
|  |  | Name of characteristics in English |  | Nom du caractère en français |  | Name des Merkmals auf Deutsch | Nombre del carácter en español |  |  |
|  |  | states of expression |  | types d'expression |  | Ausprägungsstufen | tipos de expresión |  |  |

1 Characteristic number
2 (*)
Asterisked characteristic - see Chapter 6.1.2
3 Type of expression

| QL | Qualitative characteristic | - see Chapter 6.3 |
| :--- | :--- | :--- |
| QN | Quantitative characteristic | - see Chapter 6.3 |
| PQ | Pseudo-qualitative characteristic - see Chapter 6.3 |  |

4 Method of observation (and type of plot, if applicable) MG, MS, VG, VS

- see Chapter 4.1.5

5 (+)
See Explanations on the Table of Characteristics in Chapter 8.2
6 (a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1
7 Growth stage key See Explanations on the Table of Characteristics in Chapter 8
Consult paragraph 2.3 for an explanation of the variety types.
(A): type A variety
(B): type B variety
(C): type C variety
(D): type D variety
(E): type E variety
7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

|  | English |  | français |  | deutsch | español | Example Varieties <br> Exemples <br> Beispielssorten <br> Variedades ejemplo | Note/ Nota |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1. | QL | VG |  | (a) |  |  |  |  |
|  | Leaf: variegation |  |  |  |  |  |  |  |
|  | absent |  |  |  |  |  | Aida (C), Futura 75 (A) | 1 |
|  | present |  |  |  |  |  | Divina (C) | 9 |
| 2. | QN | VG |  | (a) |  |  |  |  |
|  | Only varieties with leaf variegation: absent: Leaf: intensity of green color |  |  |  |  |  |  |  |
|  | light |  |  |  |  |  | Aida (C), Fibror 79 (A) | 1 |
|  | medium |  |  |  |  |  | Fedora 17 (A), Theresa (C) | 2 |
|  | dark |  |  |  |  |  | Finola (A), Gill (C) | 3 |
| 3. | QN | MS/VG |  | (a), (b) |  |  |  |  |
|  | Leaf: length of petiole |  |  |  |  |  |  |  |
|  | short |  |  |  |  |  | Fibrol (A), MGC 1013 (C) | 1 |
|  | medium |  |  |  |  |  | Bedrolite (C), Divina (C), Fedora 17 (A) | 2 |
|  | long |  |  |  |  |  | Carmagnola (A) | 3 |
| 4. ${ }^{*}$ ) | QN | VG |  | (a), (b) |  |  |  |  |
|  | Leaf: anthocyanin coloration of petiole |  |  |  |  |  |  |  |
|  | absent or very weak |  |  |  |  |  | Fibrol (A), Gill (C) | 1 |
|  | weak |  |  |  |  |  | Ruby (A), Theresa (C) | 2 |
|  | medium |  |  |  |  |  | Dioica 88 (A), Gayle (C) | 3 |
|  | strong |  |  |  |  |  | M-1337 (C) | 4 |
|  | very strong |  |  |  |  |  | EVLS 113 (C), Finola (A) | 5 |
| 5. (*) | QN | MS/VG | (+) | (a), (b) |  |  |  |  |
|  | Leaf: number of leaflets |  |  |  |  |  |  |  |
|  | very few |  |  |  |  |  | Bedrolite (C), MGC 1013 (C) | 1 |
|  | few |  |  |  |  |  | Aida (C), Finola (A) | 2 |
|  | medium |  |  |  |  |  | GRX53 (D), Uso 31 (A) | 3 |
|  | many |  |  |  |  |  | Fibror 79 (A) | 4 |
|  | very many |  |  |  |  |  |  | 5 |


|  | English |  | français | deutsch | español | Example Varieties <br> Exemples <br> Beispielssorten <br> Variedades ejemplo | Note/ <br> Nota |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6. | QN | MS/VG | (a), (b) |  |  |  |  |
| Central leaflet: length |  |  |  |  |  |  |  |
|  | very short |  |  |  |  | Damato Red (C) | 1 |
|  | very short to short |  |  |  |  | MGC 1013 (C) | 2 |
|  | short |  |  |  |  | Divina (C) | 3 |
|  | short to medium |  |  |  |  |  | 4 |
|  | medium |  |  |  |  | Aida (C) | 5 |
|  | medium to long |  |  |  |  |  | 6 |
|  | long |  |  |  |  | Felina 32 (A) | 7 |
|  | long to very long |  |  |  |  |  | 8 |
|  | very long |  |  |  |  | Carmagnola (A) | 9 |
| 7. (*) | QN | MS/VG | (a), (b) |  |  |  |  |
|  | Central leaflet: width |  |  |  |  |  |  |
|  | very narrow |  |  |  |  |  | 1 |
|  | very narrow to narrow |  |  |  |  | Celeste (C) | 2 |
|  | narrow |  |  |  |  | MGC 1013 (C) | 3 |
|  | narrow to medium |  |  |  |  |  | 4 |
|  | medium |  |  |  |  | Fibrol (A), Theresa (C) | 5 |
|  | medium to broad |  |  |  |  | Hulkberry (C) | 6 |
|  | broad |  |  |  |  | Gill (C), Uso 31 (A) | 7 |
|  | broad to very broad |  |  |  |  |  | 8 |
|  | very broad |  |  |  |  | Carmagnola (A), Enectabis (D) | 9 |
| 8. (*) | QN | MG/VG |  |  |  |  |  |
|  | Only varieties of type A: Time of male flowering |  |  |  |  |  |  |
|  | very early |  |  |  |  | Uso 31 (A) | 1 |
|  | very early to early |  |  |  |  |  | 2 |
|  | early |  |  |  |  |  | 3 |
|  | early to medium |  |  |  |  |  | 4 |
|  | medium |  |  |  |  | Fibrol (A) | 5 |
|  | medium to late |  |  |  |  |  | 6 |
|  | late |  |  |  |  | Felina 32 (A) | 7 |
|  | late to very late |  |  |  |  |  | 8 |
|  | very late |  |  |  |  | Dioica 88 (A) | 9 |




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|  |  | English | français | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 22. | QN | MS/VG | (c) | 2202b 2302b |  |  |  |
|  | Only varieties of types C and D: Main stem: length of internode |  |  |  |  |  |  |
|  | very short |  |  |  |  |  | 1 |
|  | very short to short |  |  |  |  | MGC 1027 (C) | 2 |
|  | short |  |  |  |  | Beatriz (C), Divina (C) | 3 |
|  | short to medium |  |  |  |  |  | 4 |
|  | medium |  |  |  |  | Aida (C), HURV2019PL (D) | 5 |
|  | medium to long |  |  |  |  | EVLS 113 (C) | 6 |
|  | long |  |  |  |  |  | 7 |
|  | long to very long |  |  |  |  |  | 8 |
|  | very long |  |  |  |  | Enectitaca (D), Obi (D) | 9 |
| 23. | QN | MS/VG | (c) | 2202 2202b 2302 2303b |  |  |  |
|  | Only varieties of types A, B and E: Main stem: thickness |  |  |  |  |  |  |
|  | thin |  |  |  |  | Finola (A) | 1 |
|  | medium |  |  |  |  | Futura 75 (A) | 2 |
|  | thick |  |  |  |  | Dioica 88 (A) | 3 |
| 24. | QN | MS/VG | (c) | 2202b 2303b |  |  |  |
|  | Only varieties of types C and D: Main stem: thickness |  |  |  |  |  |  |
|  | thin |  |  |  |  | Celeste (C) | 1 |
|  | medium |  |  |  |  | Aida (C) | 2 |
|  | thick |  |  |  |  | Obi (D) | 3 |
| 25. | QN | VG | (c) | 2202 2202b 2302 2303b |  |  |  |
|  | Only types A, D and E: Main stem: depth of grooves |  |  |  |  |  |  |
|  | shallow |  |  |  |  | Finola (A) | 1 |
|  | medium |  |  |  |  | Fedora 17 (A) | 2 |
|  | deep |  |  |  |  | Dioica 88 (A), HURV2019PL (D) | 3 |

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|  | English |  | français |  | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 28. (*) | QN | MG | (+) |  | 2204 2204b 2305 2305b |  |  |  |
|  | Inflorescence: CBD content |  |  |  |  |  |  |  |
|  | absent or very low |  |  |  |  |  | Bedrobinol (C), Enectacalm (D), Raquel (C), <br> Santhica 27 (A) | 1 |
|  | very low to low |  |  |  |  |  | Aida (C), Fedora 17 (A), Octavia (C) | 2 |
|  | low |  |  |  |  |  | Futura 75 (A), Theresa (C) | 3 |
|  | low to medium |  |  |  |  |  | Beatriz (C), Toluca (C) | 4 |
|  | medium |  |  |  |  |  | Bediol (C), Sara (C) | 5 |
|  | medium to high |  |  |  |  |  | Sibari (C) | 6 |
|  | high |  |  |  |  |  | Goya (C) | 7 |
|  | high to very high |  |  |  |  |  | A1 Philadelphia (C), Enectonica (D) | 8 |
|  | very high |  |  |  |  |  |  | 9 |
| 29. | QN | MG | (+) |  | 2204b 2305b |  |  |  |
|  | Only varieties of type C and D : Inflorescence: CBG content |  |  |  |  |  |  |  |
|  | very low |  |  |  |  |  | A1 Philadelphia (C), Bedrolite (C) | 1 |
|  | low |  |  |  |  |  | Mati (C), Moniek (C) | 2 |
|  | medium |  |  |  |  |  | HURV2019CBG (C), Juani (C), Octavia (C) | 3 |
|  | high |  |  |  |  |  | Aida (C) | 4 |
|  | very high |  |  |  |  |  |  | 5 |
| 30. | QN | VG | (+) | (c) | 2204 2202b 2306 2306b |  |  |  |
|  | Main stem: pith in cross-section |  |  |  |  |  |  |  |
|  | absent or thin |  |  |  |  |  | HURV2019PL (D), Santhica 27 (A) | 1 |
|  | medium |  |  |  |  |  | Divina (C), Fedora 17 (A) | 2 |
|  | thick |  |  |  |  |  | Finola (A), Gill (C), MGC 1009 (C) | 3 |


|  | English |  | français |  | deutsch | español | Example Varieties <br> Exemples <br> Beispielssorten <br> Variedades ejemplo | Note/ <br> Nota |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 31. | QN | MG |  |  | 22052307 |  |  |  |
|  | Seed: 1,000 seed weight |  |  |  |  |  |  |  |
|  | very low |  |  |  |  |  | Finola (A) | 1 |
|  | low |  |  |  |  |  | Chamaeleon (A), Enectitaca (D) | 2 |
|  | medium |  |  |  |  |  | Enectacalm (D), Felina 32 (A) | 3 |
|  | high |  |  |  |  |  | Santhica 27 (A) | 4 |
|  | very high |  |  |  |  |  | Fibror 79 (A) | 5 |
| 32. | PQ | VG |  |  | 22052307 |  |  |  |
|  | Seed: color of testa |  |  |  |  |  |  |  |
|  | light grey |  |  |  |  |  | Finola (A) | 1 |
|  | medium grey |  |  |  |  |  | Enectavio (D), Uso 31 (A) | 2 |
|  | grey brown |  |  |  |  |  | Enectacalm (D), Fedora 17 (A) | 3 |
|  | yellowish brown |  |  |  |  |  | Fibror 79 (A) | 4 |
|  | brown |  |  |  |  |  | Dioica 88 (A), Enectitaca (D) | 5 |
| 33. | QN | VG | (+) |  | 22052307 |  |  |  |
|  | Seed: marbling |  |  |  |  |  |  |  |
|  | weak |  |  |  |  |  | Enectacalm (D), Finola (A) | 1 |
|  | medium |  |  |  |  |  | Enectavio (D), Felina 32 (A) | 2 |
|  | strong |  |  |  |  |  | Dioica 88 (A) | 3 |

## 8. Explanations on the Table of Characteristics

### 8.1 Explanations covering several characteristics

Characteristics containing the following key in 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 (Type A) or flower senescence (Types B, C, D and E).
(b) 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 center of the plant.
(c) For type A, observations should be done on the internode below the last opposite leaves of female and/or monoecious plants. In case of types B, C, D and E, observations should be done on the internode below a fully developed leaf from the center of the plant.

### 8.2 Explanations for individual characteristics

## Ad. 5: Leaf: number of leaflets

The predominant number of leaflets in the center 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: Only varieties of type 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.


Male flower


Female flower


Longitudinal cross-section of female flower

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

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

Ad. 11: Only varieties of types B, C, D and E: Female inflorescence: intensity of anthocyanin coloration
The color of the bracts, stipules and sugarleaves ${ }^{3}$ should be observed.
3) Sugarleaves are the leaves between the clusters of female flowers.


1
absent or weak


2
medium


3
strong

## Ad. 12: Plant: proportion of monoecious plants

Cannabis sativa L . is dioecious by nature and is predominantly controlled by an XY chromosomal system, where $X X=$ female and $X Y=$ male. Monoecious plants (male and female flowers on one plant) occasionally occur naturally but are specially created by breeding activity (Bócsa, 1998). The presence of 'masculinizing' and 'feminizing' genes on the sex chromosomes further regulate sex expression, resulting in varietal variation of the proportion of male/female/monoecious plants.

Monoecious 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 | $>=96 \%$ |

## Type A:

The 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:

Feminized seed varieties may be female, monoecious, or may show a mixture of female and monoecious plants. Sex expression may be affected by environmental conditions and stress. The occurrence of a limited number of male flowers on a female flowering plant should therefore not result in labeling such plants as monoecious.

## Ad. 13: Plant: proportion of female plants

See Ad. 12

Ad. 15: Only varieties of types C and D: Female flower: length of stigmas


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


Ad. 18: Only varieties of types A, B and E: Plant: natural height
Plant height should be observed on female and/or monoecious plants from soil level to the top of the plant including inflorescence.

Ad. 19: Only varieties of types C and D: Plant: height
See Ad. 18

## Ad. 26: Only varieties of types A, B and E: Inflorescence: THC content

The method to simultaneously determine the THC, CBD, and CBG content is based on a quantitative determination of $\Delta^{9}$-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) 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 $70{ }^{\circ} \mathrm{C}$. 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 $25^{\circ} \mathrm{C}$ in a dark place.

Determination of THC/CBD/CBG 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 $25^{\circ} \mathrm{C}$ in a dark dry place.

## 2. Reagents and extraction solution

Reagents:

- $\quad \Delta^{9}$-tetrahydrocannabinol (THC), pure for chromatographic purposes.
- Cannabidiol (CBD), pure for chromatographic purposes
- Cannabigerol (CBG), 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) Equipment

- 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 $0.50 \mathrm{mg} / \mathrm{ml}$ of each of the cannabinoids in the extraction solution.
(c) Experimental conditions

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

- oven temperature

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- injector temperature
$300^{\circ} \mathrm{C}$
- detector temperature
$300^{\circ} \mathrm{C}$
(d) Injection volume: $1 \mu \mathrm{l}$

Results
THC, CBD, and CBG should be determined to two decimals in grams of $\Delta^{9}-\mathrm{THC}, \mathrm{CBD}$, and CBG resp., per 100 grams of analytical sample dried to constant weight. A tolerance of 0.03 g per 100 grams applies.

Alternative methods may be used as long as they yield the same results.

Ad. 27: Only varieties of types C and D: Inflorescence: THC content
See Ad. 26

## Ad. 28: Inflorescence: CBD content

See Ad. 26

Ad. 29: Only varieties of type C and D: Inflorescence: CBG content
See Ad. 26

Ad. 30: Main stem: pith in cross-section

1
absent or thin

2
medium

3
thick

Ad. 33: Seed: marbling
Marbling of testa: black mosaic patterns


### 8.3 Growth stages

All characteristics should be recorded at the appropriate time of plant development 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 stages (marked by *) to accommodate types $B, C, D$, and $E$ when no seed is formed. Seed formation affects the production of cannabinoids and should therefore be 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 the 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, and the third and fourth digit indicating the developmental stage of the plant.

| Code | Definition | Remarks |
| :---: | :---: | :---: |
| Germination and emergence |  |  |
| 0000 | Dry seed |  |
| 0003 | Cotyledons unfolded |  |
| Vegetative stage refers to the main stem. Leaves are considered unfolded when leaflets are at least one cm long |  |  |
| 1002 | $1^{\text {st }}$ leaf pair | 1 leaflet |
| 1004 | $2^{\text {nd }}$ leaf pair | 3 leaflets |
| 1006 | 3rd leaf pair | 5 leaflets |
| 1006b* | $5^{\text {th }}$ leaf | In vegetatively propagated plants all leaves have the same phyllotaxis |
| 10xx | Last opposite leaf pair | $x \mathrm{x}=2$ times $\mathrm{n}^{\text {th }}$ 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. <br> Perigonal bracts with no stigmas |
| 2301 | Beginning of female flowering | First stigmas visible |
| 2302 | Female flowering | $50 \%$ of bracts formed |
| $2302 b^{\star}$ | 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 |
| $2305 b^{\star}$ | Female flower senescence | $10 \%$ of stigmas brown |
| 2306 | Seed maturity | $50 \%$ of seeds hard |
| $2306 b^{*}$ | 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

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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 variety)
(...........................................)
female parent
(b) partially known cross
(please state known parent variety(ies))

female parent
(c) unknown cross
[ ]
4.1.2 Mutation
[ ]
(please state parent variety)
$\square$
4.1.3 Discovery and development
[ ]
(please state where and when discovered and how developed)
$\square$
4.1.4 Other
[ ]
(Please provide details)
$\square$

4.2 Method of propagating the variety
4.2.1 Seed-propagated varieties
(a) Cross-pollination
(b) Hybrid
(c) Feminized seed
(d) Other (please provide details)
$\square$
4.2.2 Vegetative propagation
(a) Cuttings
(b) In vitro propagation
(c) Other (state method)
$\square$
4.2.3 Other [ ]
(Please provide details)
$\square$

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 <br> (5) | Leaf: number of leafle |  |  |
|  | very few | Bedrolite (C), MGC 1013 (C) | 1[ ] |
|  | few | Aida (C), Finola (A) | 2[ ] |
|  | medium | GRX53 (D), Uso 31 (A) | 3 [ ] |
|  | many | Fibror 79 (A) | 4 [ ] |
|  | very many |  | 5 [ ] |
| 5.2 <br> (7) | Central leaflet: width |  |  |
|  | very narrow |  | 1 [ ] |
|  | very narrow to narrow | Celeste (C) | 2[ ] |
|  | narrow | MGC 1013 (C) | 3 [ ] |
|  | narrow to medium |  | 4 [ ] |
|  | medium | Fibrol (A), Theresa (C) | 5 [ ] |
|  | medium to broad | Hulkberry (C) | 6 [ ] |
|  | broad | Gill (C), Uso 31 (A) | 7 [ ] |
|  | broad to very broad |  | 8 [ ] |
|  | very broad | Carmagnola (A), Enectabis (D) | 9[] |
| 5.3 <br> (8) | Only varieties of type |  |  |
|  | very early | Uso 31 (A) | 1[ ] |
|  | very early to early |  | 2[ ] |
|  | early |  | 3 [ ] |
|  | early to medium |  | 4 [ ] |
|  | medium | Fibrol (A) | 5 [ ] |
|  | medium to late |  | 6 [ ] |
|  | late | Felina $32(\mathrm{~A})$ | 7 [ ] |
|  | late to very late |  | 8 [ ] |
|  | very late | Dioica 88 (A) | 9[] |



|  | Characteristics | Example Varieti | Note |
| :---: | :---: | :---: | :---: |
| $\begin{gathered} 5.8 \\ (18) \end{gathered}$ | Only varieties of ty |  |  |
|  | very short | Adzelviesi (A), F | 1 [ ] |
|  | very short to short |  | $2[$ ] |
|  | short |  | 3 [ ] |
|  | short to medium |  | 4 [ ] |
|  | medium | Uso 31 (A) | 5 [ ] |
|  | medium to long | Fibrol (A) | 6 [ ] |
|  | long | Felina 32 (A) | 7 [ ] |
|  | long to very long | Fibror 79 (A) | 8 [ ] |
|  | very long | Dioica 88 (A) | 9[] |
| $\begin{gathered} 5.9 \\ (19) \end{gathered}$ | Only varieties of typer |  |  |
|  | very short | MGC 1027 (C) | 1 [ ] |
|  | very short to short |  | $2[$ ] |
|  | short | Chuy (C) | 3 [ ] |
|  | short to medium |  | 4 [ ] |
|  | medium | Aida (C) | 5 [ ] |
|  | medium to long |  | 6 [ ] |
|  | long | Bedrolite (C), EV | 7 [ ] |
|  | long to very long | Obi (D) | 8 [ ] |
|  | very long |  | 9[] |
| $\begin{aligned} & 5.10 \\ & (20) \end{aligned}$ | Main stem: color |  |  |
|  | yellow | Fibror 79 (A) | 1 [ ] |
|  | medium green | Bedrobinol (C), | 2 [ ] |
|  | dark green | Aida (C), Dioica | 3 [ ] |
|  | purple | EVLS 113 (C), F | 4 [ ] |
| 5.11 <br> (26) | Only varieties of typer |  |  |
|  | absent or very low | Santhica 27 (A) | 1 [ ] |
|  | very low to low | Fedora 17 (A) | 2 [ ] |
|  | low | Futura 75 (A) | 3 [ ] |



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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 <br> variety(ies) similar to your <br> candidate variety | Characteristic(s) in which <br> your candidate variety differs <br> from the similar variety(ies) | Describe the expression of <br> the characteristic(s) for the <br> similar variety(ies) | Describe the expression of <br> the characteristic(s) for your <br> candidate variety |
| :---: | :---: | :---: | :---: |
| Example | Main stem: color (20) | yellow | medium green |

Comments:

\#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) ornamental
(e) other

| $[$ | $]$ |
| :--- | :--- |
| $[$ | $]$ |
| $[$ | $]$ |
| $[$ | $]$ |

\#
Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

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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.
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 [ ] |
| :--- | :--- |
| Yes [ ] | No [ ] |
| Yes [ ] | No [ ] |
| Yes [ ] | No [ ] |

Please provide details for where you have indicated "yes".
10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name $\square$

Signature $\square$ Date $\square$

