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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

**AGARICUS**

UPOV Code(s):

AGARI_BIS

*Agaricus bisporus* (Lange.) Sing.

## GUIDELINES

## FOR THE CONDUCT OF TESTS

## FOR DISTINCTNESS, UNIFORMITY AND STABILITY

*prepared by experts from the European Union*
*to be considered by the*
*Technical Working Party for Vegetables*
*at its fifty-first session, to be held in Roelofarendsveen, Netherlands,*
*from 2017-07-03 to 2017-07-07*

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

Alternative names:*

| *Botanical name* | *English* | *French* | *German* | *Spanish* |
|---|---|---|---|---|
| *Agaricus bisporus* (Lange.) Sing. | Mushroom | Champignon de couche | Champignon | Champiñón |

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

### ASSOCIATED DOCUMENTS

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

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

TABLE OF CONTENTS PAGE

1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Agaricus bisporus* (Lange.) Sing..

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the 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 spawn or pure culture on a suitable medium.

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

(a) 15 litres of spawn
or
(b) 2 slant tubes or agar plate (petri dish), containing a pure culture.

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

2.5 The 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 cultivations.

3.1.3 The growing cycle is normally considered to be from spawn inoculation until the end of the first flush. ~~Extension of the cultivation period can be requested by the applicant if the distinctness can only be demonstrated in the second and/or third flush.~~

TC-EDC: to clarify that all observations should be made on the first flush (if more flushes, extra characteristics should be added)
*Leading Expert: I confirm that observations should only be made in the first flush. If the current sentence in 3.1.3 "Extension of the cultivation period can be requested by the applicant if the distinctness can only be demonstrated in the second/and third flush" causes confusion, then I can agree to delete this sentence*

*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*

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.

*3.4 Test Design*

3.4.1 The design of the tests should be such that fruit bodies or parts of fruit bodies may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.4.2 ~~Each test should be designed to result in a total of at least 90 fruit bodies in the first flush, which should be divided equally over 3 replicates. 45 fruit bodies should be collected at stage 2 and 45 fruit bodies should be collected at stage 5 (see chapter 8.3).~~
New text:
*"Each test should be designed to result in a total of at least 105 fruit bodies in the first flush, which should be divided equally over 3 replicates. 45 fruit bodies should be collected at stage 2, 15 fruit bodies should be collected at stage 3, and 45 fruit bodies should be collected at stage 5 (see chapter 8.3)"*

TC-EDC: Is the described test design sufficient to observe Char. 4 Time of peak of flush?

*Leading Expert: Yes it is. Further explanation will be given in Ad.4*

TC-EDC: Additional fruit bodies have to be harvested and observed in stage 3.

*Leading Expert: Propose 105 fruit bodies instead. The new text should read:*

*"Each test should be designed to result in a total of at least 105 fruit bodies in the first flush, which should be divided equally over 3 replicates. 45 fruit bodies should be collected at stage 2, 15 fruit bodies should be collected at stage 3, and 45 fruit bodies should be collected at stage 5 (see chapter 8.3)"*

TC-EDC: It seems that it is not appropriate to refer to a total number of fruits in 3.4.2, at least not to such a small number.

*Leading Expert: I don't understand the meaning of this statement, since it is standard practice in UPOV guidelines to say how many plants are observed. Hopefully the new sentence in 3.4.2 will clarify the situation.*

3.4.3 A minimum growing surface per strain of 1m² is advised in order to obtain sufficient fruiting bodies in both stages.

*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 fruit bodies or parts of fruit bodies to be Examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single fruit bodies should be made on ~~90~~ 105 fruit bodies or parts of fruit bodies taken from each of ~~90~~ 105 fruit bodies and any other observations made on all fruit bodies in the test, disregarding any off-type fruit bodies.

TC-EDC: to clarify number of plants to be observed for Distinctness and Uniformity

*Leading Expert: 105 fruit bodies to be observed*

TC-EDC: According to 3.4.2 each characteristic is observed on 45 fruit bodies. There are at least 3 independent samples harvested in stage 2, 3 and 5.

*Leading Expert: Number of fruit bodies now revised to 105 as outlined in 3.4.2 above (45+15+45)*

4.1.5 Method of Observation

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

MG: single measurement of a group of fruit bodies or parts of fruit bodies
MS: measurement of a number of individual fruit bodies or parts of fruit bodies
VG: visual assessment by a single observation of a group of fruit bodies or parts of fruit bodies
VS: visual assessment by observation of individual fruit bodies or parts of fruit bodies

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

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

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

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

TC-EDC: to be numbered 4.2.1 and add new paragraph as 4.2.2 (see document TGP/7/5): "These Test Guidelines have been developed for the examination of cross-pollinated 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."

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 For the assessment of uniformity of ~~cross-pollinated~~ 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 ~~90~~ 45 fruit bodies (stages 2 and 5), ~~3~~ 2 off-types are allowed. In the case of a sample size of 15 fruit bodies (stage 3), 1 off-type is allowed.

TC-EDC: "cross-pollinated" to be replaced by "vegetatively"

*Leading Expert agreed*

TC-EDC: to indicate 2 off types in a sample size of 45.

*Leading Expert: Ok for me (stages 2 and 5). Furthermore, to indicate as well: "In the case of a sample size of 15 fruit bodies (stage 3), 1 off-type is allowed."*

TC-EDC: Is it appropriate to have 90?

*Leading Expert: New sample size is 105 (45+15+45). Overall text for 4.2.2 should read: "For the assessment of uniformity of vegetatively propagated varieties, a population standard of 1% and an acceptance probability of 95% should be applied. In the case of a sample size of 45 fruit bodies (stages 2 and 5), 2 off-types are allowed. In the case of a sample size of 15 fruit bodies (stage 3), 1 off-type is allowed."*

TC-EDC: If a combined *sample shall be considered, the size is probably 45+45+45=135.*

*Leading Expert: See explanation above*

*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 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) Cap: color (characteristic 8)
(b) Gills: color (characteristic 19)
(c) Basidium: spores (characteristic 21)
Time of peak of first flush (characteristic 4)
Cap: diameter (characteristic 13)

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

## 6. Introduction to the Table of Characteristics

### *6.1 Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

### *6.2 States of Expression and Corresponding Notes*

6.2.1 States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

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

| *State* | *Note* |
|---|---|
| small | 3 |
| medium | 5 |
| large | 7 |

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

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

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

### *6.3 Types of Expression*

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

### *6.4 Example Varieties*

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

*6.5 Legend*

| | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>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

## 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/<br>Nota |
|---|---|---|---|---|---|---|---|
| **1.** | | **QN VG** | **(+)** | | | | |
| | | **Mycelium: density** | **Mycélium : densité** | **Myzel: Dichte** | **Micelio: densidad** | | |
| | | weak | faible | gering | débil | J10263 | 1 |
| | | medium | moyenne | mittel | media | Sylvan A15, Horronda | 2 |
| | | strong | forte | stark | fuerte | Brawn, Heirloom | 3 |
| **2.** | | **QN VG** | **(+)** | | | | |
| | | **Number of pins** | **Nombre de tiges** | **Anzahl Knoten** | **Número de primordios** | | |
| | | few | petit | gering | bajo | Horronda | 3 |
| | | medium | moyen | mittel | medio | Amycel 2400 | 5 |
| | | many | grand | groß | alto | Sylvan A15, Horwitu | 7 |
| **3.** | **(*)** | **QN MG** | **(+)** | **2** | | | |
| | | **Time of beginning of harvest** | **Époque de début de récolte** | **Zeitpunkt des Erntebeginns** | **Época de comienzo de la cosecha** | | |
| | | early | précoce | früh | temprana | Brawn, Euromycel 30 | 3 |
| | | medium | moyenne | mittel | intermedia | Sylvan A15, Amycel 2400 | 5 |
| | | late | tardive | spät | tardía | Euromycel 58 | 7 |
| **4.** | **(*)** | **QN MG** | **(+)** | **2** | | | |
| | | **Time of peak of first flush** | **Époque du pic de la première période de pousse** | **Zeitpunkt des Höhepunktes des ersten Austriebs** | **Momento álgido del primer brote** | | |
| | | early | précoce | früh | temprano | Heirloom | 3 |
| | | medium | moyenne | mittel | intermedio | Sylvan A15, Amycel 2400 | 5 |
| | | late | tardive | spät | tardío | Brawn, Euromycel 58 | 7 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **5.** | **(*)** | **QN MS/VG** | **(a), (c)** | **2** | | | |
| | | **Stipe: length** | **Stipe : longueur** | **Stiel: Länge** | **Pie: longitud** | | |
| | | short | court | kurz | corto | Brawn | 3 |
| | | medium | moyen | mittel | mediano | Broncoh, Sylvan A15 | 5 |
| | | long | long | lang | largo | Amycel 2400, Horwitu | 7 |
| **6.** | **(*)** | **QN MS/VG** | **(+) (a)** | **2** | | | |
| TC-EDC: to check whether to have width with current states of expression or keep diameter with states “small”, “medium”, “large”<br>*Leading expert: to discuss at TWV/51* | | | | | | | |
| | | **Stipe: diameter** | **Stipe : diamètre** | **Stiel: Durchmesser** | **Pie: diámetro** | | |
| | | narrow | petit | schmal | estrecho | Somycel 53 | 3 |
| | | medium | moyen | mittel | mediano | Brawn, Broncoh | 5 |
| | | broad | grand | breit | ancho | Horronda | 7 |
| **7.** | **(*)** | **QN MS/VG** | **(+)** | **2** | | | |
| | | **Stipe: ratio length/diameter** | **Stipe : rapport longueur/diamètre** | **Stiel: Verhältnis Länge/Durchmesser** | **Pie: relación longitud/diámetro** | | |
| | | low | petit | gering | baja | Brawn | 3 |
| | | medium | moyen | mittel | media | Sylvan A15 | 5 |
| | | high | grand | hoch | alta | Somycel 53 | 7 |
| **8.** | **(*)** | **~~PQ~~ QL** **VG** | | **2** | | | |
| TC-EDC: to check whether to be indicated as QL with states “brown” and “not brown” as Chars. 9, 10 and 15 need a QL division<br><br>*Leading Expert: OK for me to indicate as QL with states “brown” and “not brown”.* | | | | | | | |
| | | **Cap: color** | **Chapeau : couleur** | **Hut: Farbe** | **Sombrero: color** | | |
| | | ~~white~~ | ~~blanc~~ | ~~weiß~~ | ~~blanco~~ | ~~Sylvan A15~~ | ~~1~~ |
| | | ~~greyish white~~ | ~~blanc grisâtre~~ | ~~gräulichweiß~~ | ~~blanco grisáceo~~ | ~~Somycel 76~~ | ~~2~~ |
| | | ~~brown~~ | ~~marron~~ | ~~braun~~ | ~~marrón~~ | ~~Amycel 2400~~ | ~~3~~ |
| | | brown | marron | braun | marrón | Amycel 2400, Somycel 76 | 1 |
| | | not brown | blanc | weiß | blanco | Sylvan A15 | 9 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **9.** | **(*)** | **QN VG** | | **2** | | | |
| | | **Only varieties with brown cap: Cap: intensity of color** | **Seulement les variétés à chapeau marron : Chapeau : intensité de la couleur** | **Nur Sorten mit braunem Hut: Hut: Intensität der Farbe** | **Solo variedades con sombrero marrón: Sombrero: intensidad del color** | | |
| | | very light | très claire | sehr hell | muy claro | Broncoh, J10263 | 1 |
| | | light | claire | hell | claro | Amycel 2400 | 3 |
| | | medium | moyenne | mittel | intermedio | Heirloom | 5 |
| | | dark | foncée | dunkel | oscuro | Brawn | 7 |
| | | very dark | très foncée | sehr dunkel | muy oscuro | BP-1 | 9 |
| **10.** | | **QL VG** | **(+)** | **2** | | | |
| | | **Only varieties with brown cap: Stipe: color** | **Seulement les variétés à chapeau marron : Stipe : couleur** | **Nur Sorten mit braunem Hut: Stiel: Farbe** | **Solo variedades con sombrero marrón: Pie: color** | | |
| | | white | blanc | weiß | blanco | Brawn, Heirloom | 1 |
| | | greyish white | blanc grisâtre | gräulichweiß | blanco grisáceo | Amycel 2400 | 2 |
| **11.** | | **QL VG** | **(+)** | **2** | | | |

TC-EDC: How is oxidation observed?

*Leading Expert: see Ad.11 below for a revised more detailed explanation*

TC-EDC: Will all varieties oxydate after a certain time?

*Leading Expert: No. The typical oxidation reaction has not been observed in several varieties, such as 'Sylvan A15', 'Horst U1', etc.*

TC-EDC: is it really QL?

*Leading Expert: Yes it is QL (see explanation above)*

TC-EDC in the explanation check whether to indicate a fix observation time

*Leading Expert: This has now been done in the revised explanation for Ad. 11 below*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Stipe: oxidation at cutting edge** | **Stipe : oxydation du bord coupé** | **Stiel: Oxidation an der Schnittkante** | **Pie: oxidación del borde de la superficie de corte** | | |
| | | absent | absente | fehlend | ausente | Sylvan A15 | 1 |
| | | present | présente | vorhanden | presente | Somycel 53, Heirloom | 9 |
| **12.** | **(*)** | **QN MS/VG** | **(a), (c)** | **2** | | | |
| | | **Cap: height** | **Chapeau : hauteur** | **Hut: Höhe** | **Sombrero: altura** | | |
| | | short | court | niedrig | bajo | J10263 | 3 |
| | | medium | moyen | mittel | mediano | Brawn, Sylvan A15 | 5 |
| | | tall | haut | hoch | alto | Euromycel 58 | 7 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **13.** | **(*)** | **QN MS/VG** | **(a), (c)** | **2** | | | |
| | | **Cap: diameter** | **Chapeau : diamètre** | **Hut: Durchmesser** | **Sombrero: diámetro** | | |
| | | small | petit | klein | pequeño | Horwitu | 3 |
| | | medium | moyen | mittel | mediano | Broncoh | 5 |
| | | large | grand | groß | grande | Sylvan A15, Heirloom | 7 |
| **14.** | **(*)** | **QN MS/VG** | **(+)** | **2** | | | |
| | | **Cap: ratio height/diameter** | **Chapeau : rapport hauteur/diamètre** | **Hut: Verhältnis Höhe/Durchmesser** | **Sombrero: relación altura/diámetro** | | |
| | | low | petit | gering | baja | Somycel 76 | 3 |
| | | medium | moyen | mittel | media | Broncoh, Sylvan A15 | 5 |
| | | high | grand | hoch | alta | Heirloom | 7 |
| **15.** | | **QL VG** | **(+)** | **2** | | | |
| | | **Only varieties with brown cap: Cap: shade of scales compared to surface** | **Seulement les variétés à chapeau marron : Chapeau : ton des écailles par rapport à la surface** | **Nur Sorten mit braunem Hut: Hut: Schattierung der Schuppen im Vergleich zur Oberfläche** | **Solo variedades con sombrero marrón: Sombrero: tono de las escamas en comparación con la superficie** | | |
| | | lighter | plus clair | heller | más claro | Amycel 2400, Heirloom | 1 |
| | | darker | plus foncé | dunkler | más oscuro | | 9 |
| **16.** | **(*)** | **QN MS/VG** | **(a), (c)** | **2** | | | |
| | | **Cap: thickness in longitudinal section** | **Chapeau : épaisseur en section longitudinale** | **Hut: Dicke im Längsschnitt** | **Sombrero: grosor en sección longitudinal** | | |
| | | thin | fin | dünn | delgado | J10263 | 3 |
| | | medium | moyen | mittel | medio | Broncoh, Horronda | 5 |
| | | thick | épais | dick | grueso | Sylvan A15 | 7 |

| | English | français | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
|---|---|---|---|---|---|---|
| **17. (*) QN VG** | | **(+)** | **2** | | | |
| | **Cap: scaling** | **Chapeau : écailles** | **Hut: Beschuppung** | **Sombrero: presencia de escamas** | | |
| | absent or very weak | absentes ou très peu nombreuses | fehlend oder sehr gering | nula o muy escasa | Somycel 53 | 1 |
| | weak | peu nombreuses | gering | escasa | Horwitu | 3 |
| | medium | moyennement nombreuses | mittel | mediana | Horronda, Heirloom | 5 |
| | strong | nombreuses | stark | abundante | Somycel 76 | 7 |
| | very strong | très nombreuses | sehr stark | muy abundante | Broncoh | 9 |
| **18. QN VG** | | **(+)** | **2** | | | |
| | **Cap: thickness of veil** | **Chapeau : épaisseur du voile** | **Hut: Dicke des Velums** | **Sombrero: grosor del velo** | | |
| | thin | fin | dünn | delgado | J10263 | 1 |
| | medium | moyen | mittel | medio | | 2 |
| | thick | épais | dick | grueso | Sylvan A15, Horronda | 3 |
| **19. (*) PQ VG** | | | **3** | | | |
| | **Gills: color** | **Lamelles : couleur** | **Lamellen: Farbe** | **Laminillas: color** | | |
| | pink | rose | pink | rosa | BP-1 | 1 |
| | light brown | marron clair | hellbraun | marrón claro | Horwitu, Horronda | 2 |
| | dark brown | marron foncé | dunkelbraun | marrón oscuro | Broncoh | 3 |
| **20. QL VG** | | **(+)** | **3** | | | |
| | **Only varieties with brown cap: Veil: annulus color** | **Seulement les variétés à chapeau marron : Voile : couleur de l'anneau** | **Nur Sorten mit braunem Hut: Velum: Farbe der Manschette** | **Solo variedades con sombrero marrón: Velo: color del anillo** | | |
| | white | blanc | weiß | blanco | Amycel 2400, Sylvan 800 | 1 |
| | brown | marron | braun | marrón | Brawn, Heirloom | 2 |

| | | English | français | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
|---|---|---|---|---|---|---|---|
| **21.** | **(*)** | **QL VG** | **(+)** | **3** | | | |
| | | **Basidium: spores** | **Basidiome : spores** | **Basidie: Sporen** | **Basidio: esporas** | | |
| | | absent | absentes | fehlend | ausentes | J10263 | 1 |
| | | present | présentes | vorhanden | presentes | Sylvan A15 | 9 |
| **22.** | | **QN MG** | | **4** | | | |
| | | **Time of cap opening** | **Époque d'ouverture du chapeau** | **Zeitpunkt der Hutöffnung** | **Época de apertura del sombrero** | | |
| | | early | précoce | früh | temprana | Horwitu | 3 |
| | | medium | moyenne | mittel | intermedia | Sylvan A15, Amycel 2400 | 5 |
| | | late | tardive | spät | tardía | Brawn, Heirloom | 7 |
| **23.** | **(*)** | **QN VG** | **(b)** | **5** | | | |
| | | **Open cap: stipe distance from base to annulus** | **Chapeau ouvert : Stipe : distance de la base à l'anneau** | **Offener Hut: Stielabstand von Basis zu Manschette** | **Sombrero abierto: distancia desde la base del pie al anillo** | | |
| | | short | courte | niedrig | corta | Amycel 2400 | 3 |
| | | medium | moyenne | mittel | mediana | Broncoh | 5 |
| | | long | longue | lang | larga | Horwitu | 7 |
| **24.** | **(*)** | **QN MS/VG** | **(b)** | **5** | | | |
| | | **Open cap: diameter** | **Chapeau ouvert : diamètre** | **Offener Hut: Durchmesser** | **Sombrero abierto: diámetro** | | |
| | | small | petit | klein | pequeño | Horwitu | 3 |
| | | medium | moyen | mittel | mediano | Broncoh, Sylvan A15 | 5 |
| | | large | grand | groß | grande | Amycel 2400, Heirloom | 7 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **25.** | **(*)** | **QN** **MS/VG** | **(b)** | **5** | | | |
| | | **Open cap: thickness** | **Chapeau ouvert : épaisseur** | **Offener Hut: Dicke** | **Sombrero abierto: grosor** | | |
| | | thin | fin | dünn | delgado | J10263 | 3 |
| | | medium | moyen | mittel | medio | Sylvan A15, Horwitu | 5 |
| | | thick | épais | dick | grueso | Brawn, Heirloom | 7 |
| **26.** | **(*)** | **QN** **VG** | **(+)** | **5** | | | |
| | | **Open cap: fraying of margin** | **Chapeau ouvert : effilochage du bord** | **Offener Hut: Ausfransen des Randes** | **Sombrero abierto: deshilachado del borde** | | |
| | | absent or weak | absent ou faible | fehlend oder gering | ausente o leve | Amycel 2400, J10263 | 1 |
| | | moderate | modéré | mäßig | moderado | Broncoh, Horwitu | 2 |
| | | strong | prononcé | stark | intenso | ML0406 | 3 |
| **27.** | **(*)** | **PQ** **VG** | **(+)** | **5** | | | |
| TC-EDC: to check whether to be indicated as QN<br>*Leading expert: to discuss at TWV/51* | | | | | | | |
| | | **Open cap: shape of central part of upper side** | **Chapeau ouvert : forme de la partie centrale de la face supérieure** | **Offener Hut: Form des mittleren Teils der Oberseite** | **Sombrero abierto: forma de la parte central de la cara superior** | | |
| | | rounded | arrondie | abgerundet | redondeada | Euromycel 58, ML1496 | 1 |
| | | plane | plane | eben | plana | Heirloom | 2 |
| | | depressed | déprimée | eingesenkt | deprimida | Broncoh | 3 |

8. Explanations on the Table of Characteristics

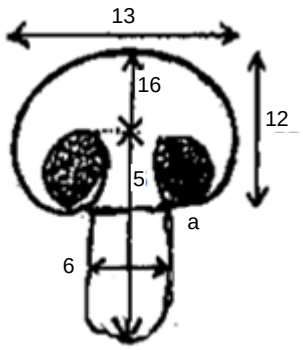
*8.1 Explanations covering several characteristics*

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

(a)

side view

longitudinal section



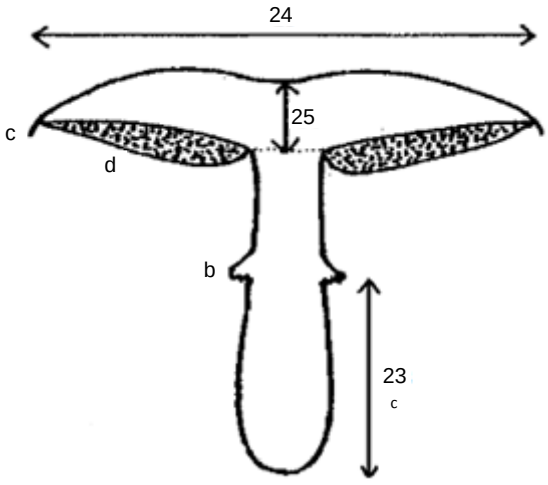

a: veil

5: Stipe: length
6: Stipe: diameter
12: Cap: height
13: Cap: diameter
16: Cap: thickness in longitudinal section

**BUTTON**

(b)

longitudinal section




b: annulus
c: cap border
d: gills

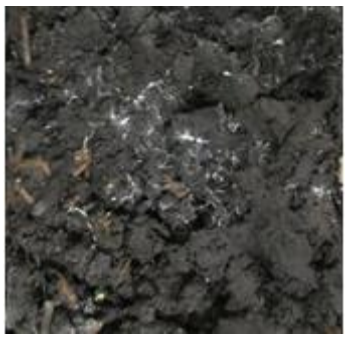
23: Stipe: distance from base to annulus
24: Open cap: diameter
25: Open cap: thickness

**FLAT/FULLY SPREAD**

(c) The fruit bodies observed at growth stage 2 should be cut longitudinally.

*8.2 Explanations for individual characteristics*

Ad. 1: Mycelium: density



1
weak

2
medium

3
strong

Ad. 2: Number of pins

A pin is a young primordial fruit body. The number of pins larger than 3 mm is visually observed 4 days after aeration.

Ad. 3: Time of beginning of harvest

The time of beginning of harvest is reached when more than 5 fruit bodies in the first flush have reached growth stage 2.

Ad. 4: Time of peak of first flush

~~The dates of harvest of fruit bodies at growth stage 2 are recorded. The time of the peak of the first flush is the time at which the largest number of fruit bodies is harvested.~~
*New text: "The dates that fruit bodies have reached growth stage 2 are recorded. The time of the peak of first flush is the time at which the largest number of fruit bodies have reached this stage."*

TC-EDC: observations seem hardly to be possible with a test design as described in 3.4.2

TC-EDC: to delete first sentence (already indicated in T.o.C)

*Leading Expert: Propose a revised explanation based upon the new wording for 3.4.2:*

*"The dates that fruit bodies have reached growth stage 2 are recorded. The time of the peak of first flush is the time at which the largest number of fruit bodies have reached this stage."*

Ad. 6: Stipe: diameter

To be observed in the middle of the stipe.

Ad. 7: Stipe: ratio length/diameter

3
low

5
medium

7
high

Ad. 10: Only varieties with brown cap: Stipe: color

The stipe color is observed at harvest.

Ad. 11: Stipe: oxidation at cutting edge

~~The stipes are cut transversally in the middle. Oxidation of the cutting edge should be observed 2 to 10 minutes after cutting.~~ *"The stipes are cut transversally in the middle. Oxidation of the cutting edge (observed visually as a yellowish to pink to red discoloration of the cut surface) should be observed 2 to 10 minutes after cutting."*

TC-EDC: How is oxidation observed?

*Leading Expert provided revised explanation: "The stipes are cut transversally in the middle. Oxidation of the cutting edge (observed visually as a yellowish to pink to red discoloration of the cut surface) should be observed 2 to 10 minutes after cutting."*

Ad. 14: Cap: ratio height/diameter

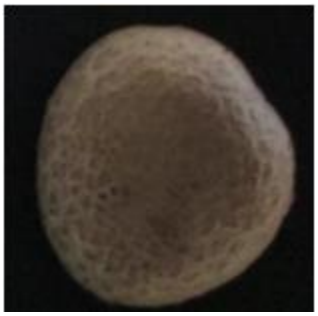
3
low

5
medium

7
high

Ad. 15: Only varieties with brown cap: Cap: shade of scales compared to surface

1
lighter



9
darker

Ad. 17: Cap: scaling

1
absent or very weak

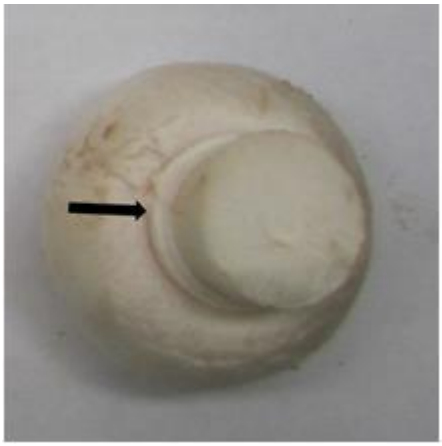
3
weak

5
medium

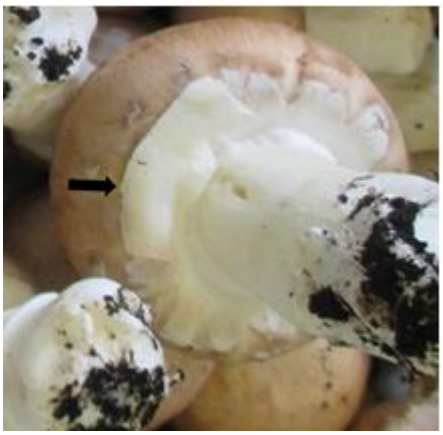
7
strong

9
very strong

Ad. 18: Cap: thickness of veil



1
thin



3
thick

Ad. 20: Only varieties with brown cap: Veil: annulus color

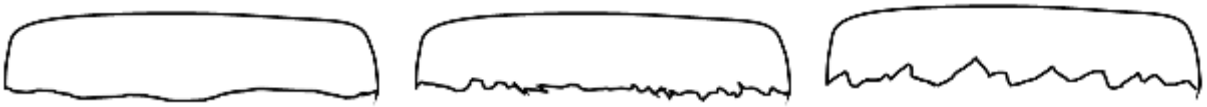
1
white

2
brown

Ad. 21: Basidium: spores

To be observed by making a sporeprint according to the methodology described by Singer (1986). If spores are formed, a sporeprint can be obtained by allowing a stage 3 fruiting body to ripen at room temperature above a sheet of white paper, which is placed below the gills. Spores of a fungal body fall onto the surface of the paper underneath. Presence of spores is revealed after two days, when a clear black-brown print on the paper has been obtained.

Ad. 26: Open cap: fraying of margin



1
absent or weak

2
moderate

3
strong

Ad. 27: Open cap: shape of central part of upper side

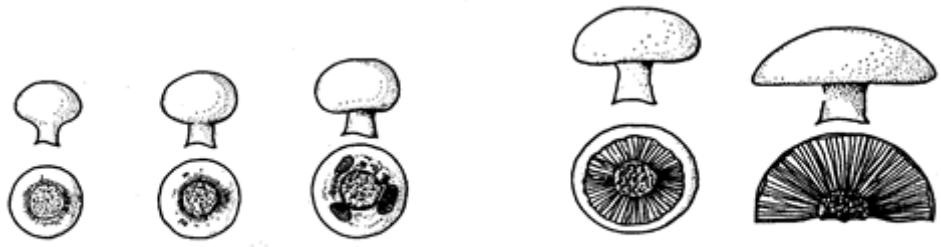
1
rounded

2
plane

3
depressed

8.3 Growth Stages and life cycle of *Agaricus bisporus*

Growth Stages

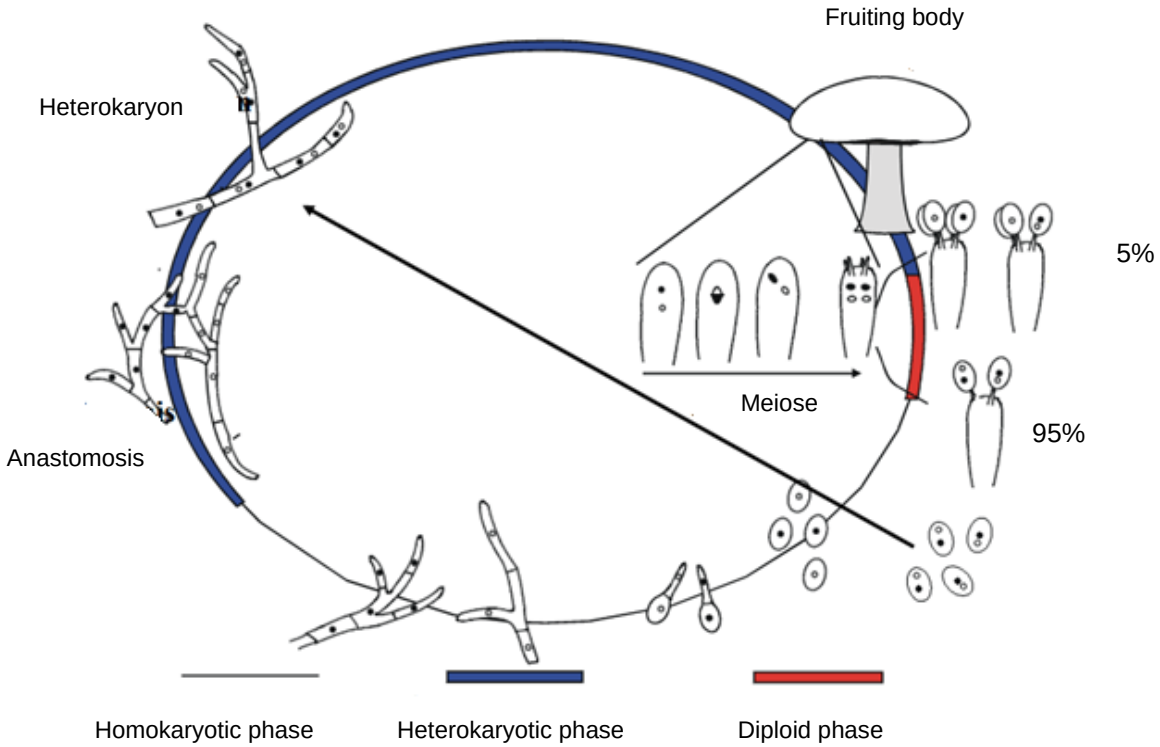


1 2 3 4 5

Explanation:

| | |
|---|---|
| 1, 2 and 3 | - button stage |
| 1 and 2 | - veil closed |
| 3 | - veil breaking |
| 4 | - opening/gills visible |
| 5 | - fully open/flat stage |

Life cycle of *Agaricus bisporus*



## 9. Literature

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Singer, R. 1986. The Agaricales in modern taxonomy, 4th edition. Koelts, Koenigstein, DE.

TC-EDC: Singer (1986) is missing – see Ad. 21

Vooren, J.G. van de, Polder, G. & Heijden, G.W.A.M. van der, 1991: Application of image analysis for variety testing of mushroom. Euphytica 57: 245-250.

Vooren, J.G. van de, Polder, G. & Heijden, G.W.A.M. van der, 1992: Identification of mushroom cultivars using image analysis. Transactions of the ASAE 35-1: 347-350.

10. Technical Questionnaire

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

| | |
|---|---|
| | Application date:<br>(not to be filled in by the applicant) |

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire

| | | |
|---|---|---|
| 1.1 | Botanical name | *Agaricus bisporus* (Lange.) Sing. |
| 1.2 | Common name | Agaricus |

2. Applicant

| | |
|---|---|
| Name | |
| Address | |
| Telephone No. | |
| Fax No. | |
| E-mail address | |
| Breeder (if different from applicant) | |

3. Proposed denomination and breeder's reference

| | |
|---|---|
| Proposed denomination (if available) | |
| Breeder's reference | |

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

#4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]

(please state parent varieties)

(b) partially known cross [ ]

(please state known parent variety(ies))

(c) unknown cross [ ]

4.1.2 Mutation [ ]

(please state parent variety)

4.1.3 Discovery and development [ ]

(please state where and when discovered and how developed)

4.1.4 Other [ ]

(please provide details)

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

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

4.2 Method of propagating the variety

4.2.1 Vegetative propagation

(a) *In vitro* propagation [ ]
(b) Other (state method) [ ]

4.2.2 Other [ ]
(Please provide details)

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

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 |
|---|---|---|---|
| TC-EDC: to review characteristics (Chars. 4 and 13 are no grouping characteristics) | | *Leading Expert: Can agree to add Chars.4 and 13 as grouping* | |
| **5.1 (4)** | **Time of peak of first flush** | | |
| | very early | | 1 [ ] |
| | very early to early | | 2 [ ] |
| | early | Heirloom | 3 [ ] |
| | early to medium | | 4 [ ] |
| | medium | Amycel 2400, Sylvan A15 | 5 [ ] |
| | medium to lated | | 6 [ ] |
| | late | Brawn, Euromycel 58 | 7 [ ] |
| | late to very late | | 8 [ ] |
| | very late | | 9 [ ] |
| **5.2 (8)** | **Cap: color** | | |
| | white | Sylvan A15 | 1 [ ] |
| | greyish white | Somycel 76 | 2 [ ] |
| | brown | Amycel 2400 | 3 [ ] |
| **5.3 (13)** | **Cap: diameter** | | |
| | very small | | 1 [ ] |
| | very small to small | | 2 [ ] |
| | small | Horwitu | 3 [ ] |
| | small to medium | | 4 [ ] |
| | medium | Broncoh | 5 [ ] |
| | medium to large | | 6 [ ] |
| | large | Heirloom, Sylvan A15 | 7 [ ] |
| | large to very large | | 8 [ ] |
| | very large | | 9 [ ] |
| **5.4 (19)** | **Gills: color** | | |
| | pink | BP-1 | 1 [ ] |
| | light brown | Horronda, Horwitu | 2 [ ] |
| | dark brown | Broncoh | 3 [ ] |
| **5.5 (21)** | **Basidium: spores** | | |
| | absent | J10263 | 1 [ ] |
| | present | Sylvan A15 | 9 [ ] |

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

6. Similar varieties and differences from these varieties

*Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.*

| Denomination(s) of variety(ies) similar to your candidate variety | Characteristic(s) in which your candidate variety differs from the similar variety(ies) | Describe the expression of the characteristic(s) for the **similar** variety(ies) | Describe the expression of the characteristic(s) for **your** candidate variety |
|---|---|---|---|
| *Example* | *Cap: color* | *greyish white* | *brown* |
| | | | |
| | | | |
| | | | |

Comments:

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

#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

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

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

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 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 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 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 material to be examined has been subjected to:

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

Please provide details for where you have indicated “yes”.

.....

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

Applicant’s name

Signature Date

[End of document]