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

# **DRAFT**

#### **SHIITAKE**

UPOV Code: LENTI ELO

JP: Botanical name has been checked Lentinula edodes (Berk.) Pegler

(Botanical name and UPOV code to be considered in conjunction with document TWV/44/5)

#### **GUIDELINES**

#### FOR THE CONDUCT OF TESTS

#### FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by an expert from Japan

to be considered by

the Technical Working Party for Vegetables at its forty-fourth session, to be held in Veliko Tarnovo, Bulgaria, from July 5 to 9, 2010

Alternative Names:\*

| Botanical name                     | English                   | French | German | Spanish |
|------------------------------------|---------------------------|--------|--------|---------|
| Lentinula edodes<br>(Berk.) Pegler | Shiitake,<br>Oak Mushroom |        |        |         |

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.

<sup>\*</sup> 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. 2. 3. Number of Growing Cycles 3 3.2 Testing Place 3 ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY......4 4.2 Uniformity 6 4.3 Stability 6 6.3 Types of Expression......8 TABLE OF CHARACTERISTICS/TABLEAU DES CARACTERES/MERKMALSTABELLE/TABLA DE CARACTERES......9 EXPLANATIONS ON THE TABLE OF CHARACTERISTICS ......18 8.

#### **Comments**

UA: Ukraine HU: Hungary JP: Japan

Highlighting: amendments in accordance with document TGP/7/2

- 3 -

#### 1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Lentinula edodes* (Berk.) Pegler (Marasmiaceae).

#### 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 and as a pure culture on a suitable medium.
- 2.3 The minimum quantity of material, to be supplied by the applicant, should be:

1 liter of spawn and 3 slant tubes containing a pure culture.

HU: we propose 2 liters spawn JP: agree to proposal

- 2.4 If spawn is delivered it should be of a quality which ensures that all relevant characteristics of the variety will be expressed. In particular, mycelium on grain should be visible to the naked eye, the grain should not be colonized to such an extent that kernels stick together. The spawn should not be older than 3 months and having been stored under proper conditions.
- 2.5 If pure cultures are delivered, they must be shipped on slant agar tubes with appropriate medium such as PDA (potato dextrose agar) or Melt extract agar. Tubes should be covered by cotton plugs or silicon plugs or plastic caps allowing sterile air diffusion. Cultures should be fresh, i.e. not stored for longer than 2 weeks at low temperature.

#### 3. Method of Examination

#### 3.1 Number of Growing Cycles

The minimum duration of tests should normally be a single growing cycle (two harvestings).

HU: at bed-log cultivation. Two growing cycle at sawdust cultivation (harvesting only the first flush).

#### 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".

- 4 -

#### 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 recommended method of observing the characteristic is indicated by the following key in the second column of the Table 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.

#### 3.4 Test Design

- 3.4.1 Each test should be designed to result in a total of at least 75 wood logs or 75 sawdust blocks, which should be divided between at least two replicates.
- 3.4.2 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.

#### 3.5 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.

- 5 -

#### 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 / Parts of fruit bodies to be Examined

Unless otherwise indicated, all observations for the purposes of distinctness should be made on at least 50 fruit bodies or parts taken from each of 50 fruit bodies, disregarding any off-type plants.

#### 4.1.5 Method of Observation

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

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

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

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

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

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

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

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

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

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

#### 4.2 Uniformity

- 4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:
- 4.2.2 For the assessment of uniformity, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of [(HU: 100 = 50 (see 3.4)] fruit bodies, [HU: 3 = 2] off-types are allowed.

#### 4.3 Stability

- 4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.
- 4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new 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:

#### JP: propose to add following grouping char. (only TQ)

- ( ) Spawn: cultivation type (1: bed-log cultivation 2: saw-dust cultivation)
- (a) Cap: shape of vertical section (characteristic 10)
- (b) Cap: color of upper side (characteristic 12)
- (c) Cap: distribution of scale (characteristic 15)
- (d) Gill: shape (characteristic 18)
- (e) Stipe: shape (characteristic 23)
- 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. <u>Introduction to the Table of Characteristics</u>

#### 6.1 Categories of Characteristics

#### 6.1.1 Standard Test Guidelines Characteristics

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

#### 6.1.2 Asterisked Characteristics

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

#### 6.2 States of Expression and Corresponding Notes

- 6.2.1 States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.
- 6.2.2 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

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

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

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

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

#### 6.3 Types of Expression

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

#### 6.4 Example Varieties

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

- 6.5 Legend
- (\*) Asterisked characteristic see Chapter 6.1.2
- QL Qualitative characteristic see Chapter 6.3
- QN Quantitative characteristic see Chapter 6.3
- PQ Pseudo-qualitative characteristic see Chapter 6.3

Type of example variety belong to:

(B) = Bed-log cultivation type, (S) = Sawdust cultivation type

#### JP: Explanation of Cultivation Type

<u>Bed-log cultivation type:</u> Group of varieties that is suited to bed-log cultivation. And group of varieties that can be grown only by bed-log cultivation.

<u>Sawdust cultivation type:</u> Group of varieties that is suited to sawdust cultivation. Bed-log and sawdust both cultivation type that can be grown are included in the sawdust cultivation type.

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

|                   |            | English                                       | français                          | deutsch | español  | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|-------------------|------------|---|-----------------------------------|---------|--|---|---------------|
| 1.                | VG         | Hyphae: density                               |                                   |         |  |   |               |
| QN                | (a)        | sparse  |                                   |         |  | HS607, Mori XR1   | 1             |
|                   |            | intermediate                                  |                                   |         |  | Morino Natsumi  | 2             |
|                   |            | dense   |                                   |         |  | KX-S055   | 3             |
| 2. (+)            | VG         | Aerial hyphae:<br>development                 |                                   |         |  |   |               |
| QN                | (a)        | weak  |                                   |         |  | HS607   | 3             |
|                   |            | medium  |                                   |         |  | Morino Natsumi,<br>KX-S055  | 5             |
|                   |            | strong  |                                   |         |  | KX-S005   | 7             |
| <b>3.</b> (*)     | VG         | Colony: color of surface                      |                                   |         |  |   |               |
| QL                | (a)        | white   |                                   |         |  | Morino Natsumi,<br>Mori XR1   | 1             |
|                   |            | brownish                                      |                                   |         |  | HS607, KX-S055  | 2             |
| <b>4.</b> (*) (+) | MS         | Mycelia: optimum<br>temperature for<br>growth |                                   |         |  |   |               |
| QN                | (b)        | Lower than 23°C                               |                                   |         |  |   | 1             |
|                   |            | 23°C  |                                   |         |  | Kinko 243   | 3             |
|                   |            | 25°C  |                                   |         |  | Kinko 115, HS607,<br>Mori XR1   | 5             |
|                   |            | 27°C  |                                   |         |  | Morino Natsumi  | 7             |
|                   |            | higher than 27°C                              |                                   |         |  |   | 9             |
| 5.<br>(*)<br>(+)  | MS         | Mycelia: growth rate at 10°C                  | be established i<br>which tempera |         | optimum temperature w<br>is not important to know<br>growing slower. |   |               |
| QN                | <b>(b)</b> | slow  |                                   |         |  |   | 3             |
|                   |            | medium  |                                   |         |  | HS607   | 5             |
|                   |            | fast  |                                   |         |  | KX-S055   | 7             |

## TG/SHIITK(proj.2) Shiitake, 2010-05-21 - 10 -

|                   |            | English                                      | français | deutsch | español | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|-------------------|------------|--|----------|---------|---------|---|---------------|
| 6.<br>(*)<br>(+)  | MS         | Mycelia: growth<br>rate at 15°C              |          |         |         |   |               |
| QN                | <b>(b)</b> | slow   |          |         |         |   | 3             |
|                   |            | medium                                       |          |         |         | Susono 360, HS607   | 5             |
|                   |            | fast   |          |         |         | Yujiro  | 7             |
| 7.<br>(*)<br>(+)  | MS         | Mycelia: growth<br><mark>rate</mark> at 20°C |          |         |         |   |               |
| QN (              | <b>(b)</b> | slow   |          |         |         |   | 3             |
|                   |            | medium                                       |          |         |         | Morino Natsumi  | 5             |
|                   |            | fast   |          |         |         |   | 7             |
| 8.<br>(*)<br>(+)  | MS         | Mycelia: growth<br>rate at 25°C              |          |         |         |   |               |
| QN                | <b>(b)</b> | slow   |          |         |         |   | 3             |
|                   |            | medium                                       |          |         |         | Susono 360, HS73  | 5             |
|                   |            | fast   |          |         |         |   | 7             |
| 9.<br>(*)<br>(+)  | MS         | Mycelia: growth<br>rate at 30°C              |          |         |         |   |               |
| QN                | <b>(b)</b> | slow   |          |         |         |   | 3             |
|                   |            | medium                                       |          |         |         | Kinko 115   | 5             |
|                   |            | fast   |          |         |         | Morino Natsumi  | 7             |
| 10.<br>(*)<br>(+) | VG         | Cap: shape of vertical section               |          |         |         |   |               |
| PQ                | (c)        | umbilicate                                   |          |         |         |   | 1             |
|                   |            | flat   |          |         |         |   | 2             |
|                   |            | convex                                       |          |         |         |   | 3             |
|                   |            | umbonate                                     |          |         |         |   | 4             |

# TG/SHIITK(proj.2) Shiitake, 2010-05-21 - 11 -

|               |           | English                                   | français      | deutsch                                      | español  | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota          |
|---------------|-----------|---|---------------|--|----------|---|------------------------|
| 11.<br>(*)    | VG/<br>MS | Cap: diameter                             | UA: For chara | acteristic 11: QN inste                      | ad of PQ |   |                        |
| (+)           | (c)       | small                                     |               |  |          | Morino Harumitsu  | 3                      |
| <del>PQ</del> |           | medium                                    |               |  |          | HS73  | 5                      |
| QN            |           | large                                     |               |  |          |   | 7                      |
| 12.<br>(*)    | VG        | Cap: color of upper side                  |               |  |          |   |                        |
| PQ            | (c)       | white                                     |               |  |          | Kinko 989   | 1                      |
|               |           | yellowish brown                           |               |  |          | HS73  | 2                      |
|               |           | brown                                     |               |  |          | Susono 360, HS607   | 3                      |
|               |           | reddish brown                             |               |  |          | Akiyama A-526   | 4                      |
| 13.<br>(*)    | MS        | Cap: thickness                            |               |  |          |   |                        |
| (+)           | (c)       | thin                                      |               |  |          | Morino Harumitsu  | 3                      |
| QN            |           | medium                                    |               |  |          | Susono 360, Morino<br>Natsumi, KX-S055                                    | 5                      |
|               |           | thick                                     |               |  |          | Akiyama A-526   | 7                      |
| 14.           | VS        | Cap: hardness                             |               | acteristic 14: QL inste<br>be indicate as PQ | ad of QN |   |                        |
| <b>QN</b>     | (c)       | soft=>softness                            |               |  |          |   | 3 <mark>=&gt;1</mark>  |
| PQ            |           | medium <mark>=&gt;</mark><br>intermediate |               |  |          | Susono 360, HS607,<br>KX-S055   | 5 <mark>=&gt;</mark> 2 |
|               |           | hard <mark>=&gt;solidity</mark>           |               |  |          | Akiyama A-526   | 7 <mark>=&gt;</mark> 3 |

|                       |     | English                    | français          | deutsch   | español   | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|-----------------------|-----|----------------------------|-------------------|---|---|---|---------------|
| 15.<br>(*)<br>(+)     | VG  | Cap: distribution of scale | HU: How can type? | we establish the size o                         | of the scale (char.16) at the                     | nis   |               |
| QL                    | (c) | whole                      |                   |   |   | Akiyama A-526   | 1             |
|                       |     | periphery                  |                   |   |   | Susono 360,<br>Morino Natsumi,<br>KX-S055                                 | 2             |
| <b>16.</b> (+)        | VG  | Cap: size of scale         |                   | ecessary, because the<br>s difficult. The examp | observation of this<br>le varieties are almost th | ne  |               |
| QN                    | (c) | small                      |                   |   |   | HS73  | 3             |
|                       |     | medium                     |                   |   |   | Susono 360,<br>Morino Natsumi   | 5             |
|                       |     | large                      |                   |   |   | Kinko 169   | 7             |
| 17.<br><del>(+)</del> | VG  | Cap: color of scale        | UA: Character     | ristics 17: without (+)                         |   |   |               |
| PQ                    | (c) | white                      |                   |   |   | Susono 360,<br>Morino Natsumi,<br>Mori XR1                                | 1             |
|                       |     | brownish                   |                   |   |   | Yujiro, HS607   | 2             |
| 18.<br>(*)<br>(+)     | VG  | Gill: shape                |                   |   |   |   |               |
| PQ                    | (c) | apart from stipe           |                   |   |   | Kinko 115, HS607,<br>KX-S055  | 1             |
|                       |     | attached to stipe          |                   |   |   | Morino Natsumi,<br>Mori XR1   | 2             |
| 19.<br>(*)<br>(+)?    | VG  | Gill: arrangement of row   | ' UA: Character   | ristics 19: without (+)                         |   |   |               |
| QL                    | (c) | straight                   |                   |   |   | Kinko 115,<br>Morino Natsumi,<br>KX-S055                                  | 1             |
|                       |     | ripple or crinkle          |                   |   |   | Akiyama A-526,<br>Mori XR1  | 2             |

| - 1 | 1 | _ |
|-----|---|---|

|                   |     | English             | français         | deutsch              | español  | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|-------------------|-----|---------------------|------------------|----------------------|----------|---|---------------|
| 20.               | VG  | Gill: width         |                  |                      |          |   |               |
| (+)               |     |                     |                  |                      |          |   |               |
| QN                | (c) | narrow              |                  |                      |          | Yujiro  | 3             |
|                   |     | medium              |                  |                      |          | Mori 505  | 5             |
|                   |     | wide                |                  |                      |          |   | 7             |
| 21.               | VG  | Gill: density       | UA: For characte | eristic 21: QL inste | ad of QN |   |               |
| <u>(+)</u>        |     |                     |                  |                      |          |   |               |
| <del>QN</del>     | (c) | sparse              |                  |                      |          |   | 1             |
| PQ                |     | intermediate        |                  |                      |          | Yujiro  | 2             |
|                   |     | dense               |                  |                      |          | Kinko 115,<br>Morino Natsumi,<br>KX-S055                                  | 3             |
| 22.               | VG  | Gill: color         |                  |                      |          |   |               |
| (+)               |     |                     |                  |                      |          |   |               |
| PQ                | (c) | white               |                  |                      |          | Kinko 115,<br>Morino Natsumi,<br>Mori XR1                                 | 1             |
|                   |     | light yellow        |                  |                      |          | HS607, KX-S055  | 2             |
|                   |     | light yellow orange |                  |                      |          | HS73  | 3             |
|                   |     | light brown         |                  |                      |          |   | 4             |
| 23.<br>(*)<br>(+) | VG  | Stipe: shape        | UA: For characte | eristic 23: QL inste | ad of PQ |   |               |
| <del>PQ</del>     | (c) | cylindrical         |                  |                      |          | Kinko 115, Morino<br>Natsumi, KX-S055                                     | 1             |
| <b>QL</b>         |     | thick to upper part |                  |                      |          | Susono 360  | 2             |
|                   |     | thick to lower part |                  |                      |          | JMS 7H-1  | 3             |

# TG/SHIITK(proj.2) Shiitake, 2010-05-21 - 14 -

|                       |     | English               | français                         | deutsch | español                     | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|-----------------------|-----|-----------------------|----------------------------------|---------|-----------------------------|---|---------------|
| 24.<br>(*)<br>(+)     | MS  | Stipe: length         |                                  |         |                             |   |               |
| QN                    | (c) | very short            |                                  |         |                             | Yujiro  | 1             |
|                       |     | short                 |                                  |         |                             |   | 3             |
|                       |     | medium                |                                  |         |                             |   | 5             |
|                       |     | long                  |                                  |         |                             | Akiyama A-526   | 7             |
|                       |     | very long             |                                  |         |                             |   | 9             |
| 25.<br>(*)<br>(+)     | MS  | Stipe: thickness      |                                  |         |                             |   |               |
| QN                    | (c) | thin                  |                                  |         |                             | Morino Natsumi  | 3             |
|                       |     | medium                |                                  |         |                             | HS607   | 5             |
|                       |     | thick                 |                                  |         |                             |   | 7             |
| <b>26.</b> (*)        | VG  | Stipe: color          |                                  |         |                             |   |               |
| PQ                    | (c) | white                 |                                  |         |                             | Mori XR1  | 1             |
|                       |     | brownish              |                                  |         |                             | Kinko 115, Morino<br>Natsumi, KX-S055                                     | 2             |
| 27.<br>(*)<br>(+) ?   | VG  | Stipe: fluff          | UA: Character<br>presence of (+) |         | s are absent but there is a |   |               |
| QL                    | (c) | absent                |                                  |         |                             | Kinko 989   | 1             |
|                       |     | present               |                                  |         |                             | Kinko 115,<br>Morino Natsumi,<br>KX-S055                                  | 9             |
| 28.<br><del>(+)</del> | VG  | Stipe: color of fluff | UA: Character presence of (+)    |         | s are absent but there is a |   |               |
| <del>PQ</del>         | (c) | white                 |                                  |         |                             | Mori XR1  | 1             |
| <b>QL</b>             |     | brownish              |                                  |         |                             | Kinko 115, Morino<br>Natsumi  | 2             |

|                       |     | English   | français                         | deutsch  | español                   | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note,<br>Nota          |
|-----------------------|-----|---|----------------------------------|--|---------------------------|---|------------------------|
| 29.                   | VG  | Stipe: hardness   |                                  | ecteristic 29 is this QL<br>be indicated as PQ | ? Solidity or softness?   |   |                        |
| QN                    | (c) | soft=>softness  |                                  |  |                           |   | 3 <mark>=&gt;1</mark>  |
| PQ                    |     | medium<br>=>intermediate  |                                  |  |                           | Susono 360, HS607,<br>Mori XR1  | 5 <mark>=&gt;</mark> 2 |
|                       |     | hard <mark>=&gt;solidity</mark>                                 |                                  |  |                           |   | 7 <mark>=&gt;</mark> 3 |
| <b>30.</b> (+)        | MS  | Fruit body: ratio of<br>diameter of cap /<br>length of stipe    |                                  |  |                           |   |                        |
| QN                    | (c) | very small  |                                  |  |                           | Kinko 610   | 1                      |
|                       |     | small   |                                  |  |                           | Mori 252  | 3                      |
|                       |     | medium  |                                  |  |                           | Susono 360, Akiyama<br>A-526  | 5                      |
|                       |     | large   |                                  |  |                           | Morino Natsumi  | 7                      |
|                       |     | very large  |                                  |  |                           | Morino Harumitsu  | 9                      |
| 31.<br><del>(+)</del> | MS  | Fruit body: ratio of<br>diameter of cap /<br>thickness of stipe | UA: Character<br>presence of (+) |  | are absent but there is a |   |                        |
| QN                    | (c) | very small  |                                  |  |                           |   | 1                      |
|                       |     | small   |                                  |  |                           | ML12  | 3                      |
|                       |     | medium  |                                  |  |                           | JHS KV92  | 5                      |
|                       |     | large   |                                  |  |                           | Morino Harumitsu  | 7                      |
|                       |     | very large  |                                  |  |                           | Mori 4T98   | 9                      |
| <b>32.</b> (+)        | MS  | Fruit body: dry<br>weight                                       |                                  |  |                           |   |                        |
| QN                    | (c) | light   |                                  |  |                           | HS73  | 3                      |
|                       | \-J | medium  |                                  |  |                           | Susono 360,<br>Akiyama A-526  | 5                      |
|                       |     | heavy   |                                  |  |                           | <del>-</del>  |                        |

|                           |     | English   | français  | deutsch  | español  | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|---------------------------|-----|---|---|--|--|---|---------------|
| 33.                       | MS  | Fruit body: ratio of<br>oven-dry weight /<br>fresh weight |   |  |  |   |               |
| QN                        | (c) | light   |   |  |  | Morino Natsumi  | 3             |
|                           |     | medium  |   |  |  |   | 5             |
|                           |     | heavy   |   |  |  |   | 7             |
| 34.                       | VG  | Fruit body: type of fruiting                              | presence of (+)<br>HU: 34 and 36 is<br>delete 34. If 34 v | s almost the same. V<br>vill be kept, explana            | s are absent but there is a  Ve propose to keep 36. and tion is necessary.  and to add explanation |   |               |
| PQ                        | (c) | short term<br>=> aggregate                                |   |  |  | Susono 360,<br>Morino Natsumi,<br>KX-S055                                 | 1             |
|                           |     | long term => scattering                                   |   |  |  | Yujiro, ML8   | 2             |
| 35.<br>(*)<br>(+)?<br>(+) | VG  | Fruit body: period from inoculation to fruiting induction | there is a present<br>HU: fruiting ind                    | ce of (+)<br>luction means appea<br>perature reduction ( | ample stains are absent but<br>arance of primordium or the<br>e.g. by water treatment)?            | 2   |               |
| QN                        |     | short   |   |  |  | HS73(S),<br>Kinko 702(B),<br>A-555(B)                                     | 3             |
|                           |     | medium  |   |  |  | HS608(B),<br>S-035(B),<br>Kinko 697(B)                                    | 5             |
|                           |     | long  |   |  |  | Kinko 169(B),<br>Mori-yujiro(B),<br>HS705(S),<br>S-035(S),<br>ML8(S)      | 7             |

### TG/SHIITK(proj.2) Shiitake, 2010-05-21 - 17 -

|            |    | English   | français             | deutsch   | español | Example Varieties/<br>Exemples/<br>Beispielssorten/<br>Variedades ejemplo | Note/<br>Nota |
|------------|----|---|----------------------|---|---------|---|---------------|
| 36.<br>(+) | VG | Fruit body: period from fruiting induction to harvest | characteristic in TQ | 66: presence of asteris<br>acteristics 36 example<br>xplanation |         |   |               |
| QN         |    | short   |                      |   |         | KX-S055(B),<br>A-555(B),<br>HS-73(S),<br>S-005(S)                         | 3             |
|            |    | medium  |                      |   |         | A-526(B),<br>HS72(S),<br>HS705(S)   | 5             |
|            |    | long  |                      |   |         | ML8(B),<br>ML8(S),<br>S-035(S)  | 7             |

#### 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) <u>Hyphae and Colony</u>: Hyphae and Colony should be observed by pure cultures.

Type of medium: PDA

Plate: 9cm in inside diameter and 2cm in height

Conditions : in the dark at  $25 \pm 2$ °C

Observations: (hyphae) developed to 70% of the diameter of the plate

(colony) 14 days after

Number of plate: more than two

(b) Mycelia: Mycelia should be observed by pure cultures.

Type of medium: PDA

Tube/Plate: growth tube or 9cm in inside diameter and 2cm in height

Conditions: in the dark at specified temperature

Observations: 14 days after [HU: 14 days also at 10°C?]

Number of tube: more than five

(c) <u>Stipe, cap and gills</u>: Unless otherwise indicated, all characteristics of the fruit bodies, the cap, the stipe and the gills should be recorded at harvest maturity (stage 4[see additional information] hand picked mushrooms; freshly harvested).

#### 8.2 Explanations for individual characteristics

#### Ad. 2: Aerial hyphae: development



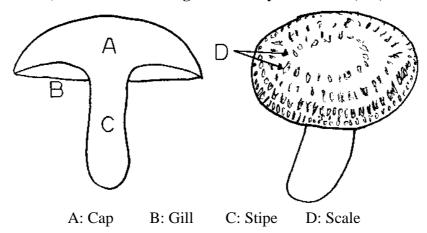
Observe density and height of aerial hyhae

# Ad. 4 Mycelia: optimum temperature for growth Ad. 5, 6, 7, 8, 9: Mycelia: growth rate at 10°C, 15°C, 20°C, 25°C, 30°C

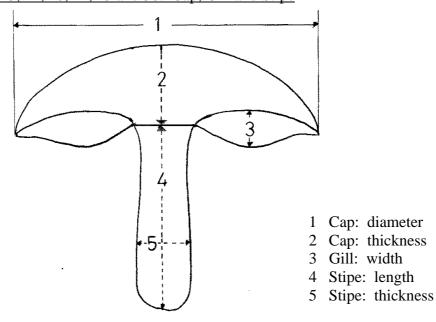
Measure the length [HU: or diameter (see 8.1b tube/plate)] that grew up on the 14<sup>th</sup> day from the 4<sup>th</sup> day of mycelia cultured at each temperature. The incubation temperature of mycelia that grow up longest becomes an optimum temperature. Amount of Mycelia growth per day at each temperature are considered to be the growth rate.

#### Ad. 10, 11, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 25 and 27: Cap, Gill and Stipe

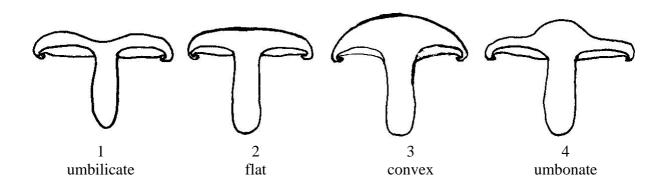
HU: Char.10, 11, 13, 15, 18, 20, 23, 24, 25 has own drawing/photo. It is not necessary the same (e.g. see below). But other drawing is necessary for char.16, 21, 27.



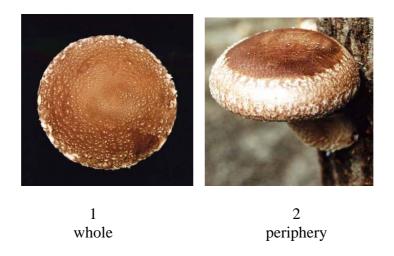
Ad. 11, 13, 14, 20, 24, 25 and 30: Cap, Gill and Stipe



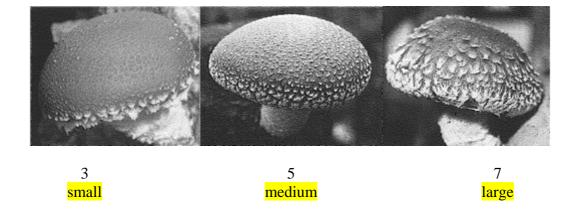
# Ad. 10: Cap: shape of vertical section



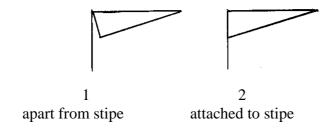
Ad. 15: Cap: distribution of scale



Ad. 16: Cap: size of scale



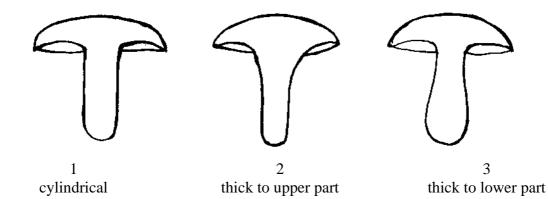
## Ad. 18: Gill: shape



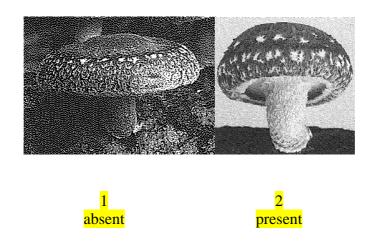
## Ad. 21: Gill: density

(The photos are scheduled to be prepared.)

Ad. 23: Stipe: shape



# Ad. 27 Stipe: fluff



#### Ad. 32: Fruit body: dry weight

The fruiting body dries at 60°C until becoming a constant weight.

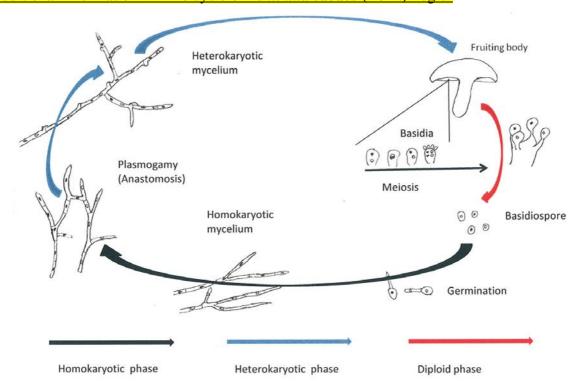
#### Ad. 34: Fruit body: type of fruiting

Growth habit of fruiting under suitable cultivated condition. Fruiting are aggregated in a short term or scattered in a long term.

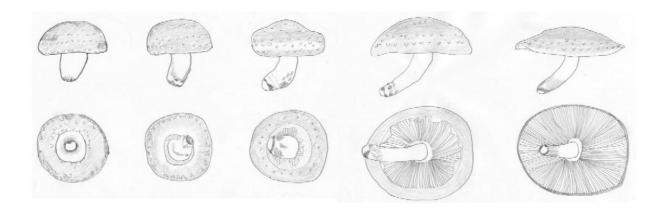
Ad. 35: Fruit body: period from inoculation to fruiting induction Ad. 36: Fruit body: period from fruiting induction to harvest

In the fruiting induction, there are water soaking treatment, water sprinkling treatment, low temperature treatment, and physical treatment. These methods should be treated at suitable time.

#### Additional information 1: Life cycle of Lentinula edodes (Berk.) Pegler



# Additional information 2: Stage of fruit body



1 2 3 4 5

Explanation: 1 and 2 - veil closed 4 - 80 to 90% open / gills visible 3 - veil breaking 5 - fully open

TG/SHIITK(proj.2) Shiitake, 2010-05-21 - 24 -

## 9. Literature

Pegler, D.N., 1975 (1976): The classification of the genus Lentinus Fr. (Basidiomycota), Kavaka 3:11-20

Ministry of Agriculture, Forestry and Fisheries, 1996: National Test Guideline for Shiitake, JP

Kirk, P.M., Cannon P.F., Minter D.W. and Stalpers J.A. (eds.), 2008: Dictionary of the Fungi 10th edition, CAB International, UK ISBN 978-0-85199-826-8

# 10 <u>Technical Questionnaire</u>

| TECHNICAL QUESTIONNAIR |                                      |       | Page {x} of {y}                           | Reference Number:  |
|------------------------|--------------------------------------|-------|---|--|
|                        |                                      |       |   | Application date: (not to be filled in by the applicant) |
|                        |                                      |       | NICAL QUESTIONN<br>ion with an applicatio | JAIRE<br>n for plant breeders' rights                    |
| 1.                     | Subject of the Technical Qu          | esti  | onnaire                                   |  |
|                        | 1.1 Botanical Name                   | Len   | tinula edodes (Berk.)                     | Pegler   |
|                        | 1.2 Common Name                      | Shii  | itake                                     |  |
| 2.                     | Applicant                            |       |   |  |
|                        | Name                                 |       |   |  |
|                        | Address                              |       |   |  |
|                        | Telephone No.                        |       |   |  |
|                        | Fax No.                              |       |   |  |
|                        | E-mail address                       |       |   |  |
|                        | Breeder (if different from ap        | pplic | cant)                                     |  |
|                        | , <u>L</u>                           |       |   |  |
| 3.                     | Proposed denomination and            | bre   | eder's reference                          |  |
|                        | Proposed denomination (if available) |       |   |  |
|                        | Breeder's reference                  |       |   |  |

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

| #4. Inf                                  | 'amatian | on the broading scheme and muone action of the variety                                   |
|--|----------|--|
|  |          | on the breeding scheme and propagation of the variety                                    |
| 4.1                                      |          | ng scheme  |
|  | Variet   | y resulting from:  |
|  | 4.1.1    | Crossing   |
|  |          | (a) controlled cross [ ] (please state parent varieties)                                 |
| innum il                                 | (        | female parent male parent  |
|  |          | (b) partially known cross [ ] (please state known parent variety(ies))                   |
| hannannannannannannannannannannannannann | (        | female parent  |
|  |          | (c) unknown cross [ ]  |
| à manana                                 | 4.1.2    | Mutation [ ] (please state parent variety)   |
| i.                                       | 4.1.3    | Discovery and development [ ] (please state where and when discovered and how developed) |
| mananani                                 | 4.1.4    | Other [ ] (please provide details)   |
| homomomomomomomomomomomomomomomomomomom  |          |  |
| 4.2                                      | Metho    | d of propagating the variety   |
|  | 4.2.1    | Vegetative propagation   |
|  | (        | a) cuttings [ ] b) in vitro propagation [ ] c) other (state method) [ ]                  |

 $<sup>^{\#}</sup>$  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: |
|-------------------------|-------------------------|-------------------|

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            |
|----------------|---|-------------------|-----------------|
| <del>5.</del>  | Spawn: cultivation type                                     |                   |                 |
|                | bed-log cultivation type                                    |                   | <del>1[ ]</del> |
|                | saw dust cultivation type (including both cultivation type) |                   | <del>2[_]</del> |
| <b>5.1</b> (7) | Mycelia: growth at 20°C                                     |                   |                 |
|                | very slow   |                   | 1[]             |
|                | very slow to slow   |                   | 2[]             |
|                | slow  |                   | 3[]             |
|                | slow to medium  |                   | 4[]             |
|                | medium  | Morino Natsumi    | 5[]             |
|                | medium to fast  |                   | 6[]             |
|                | fast  |                   | 7[]             |
|                | fast to very fast   |                   | 8[]             |
|                | very fast   |                   | 9[]             |
| <b>5.2</b> (9) | Mycelia: growth at 30°C                                     |                   |                 |
|                | very slow   |                   | 1[]             |
|                | very slow to slow   |                   | 2[]             |
|                | slow  |                   | 3[]             |
|                | slow to medium  |                   | 4[]             |
|                | medium  | Kinko 115         | 5[]             |
|                | medium to fast  |                   | 6[]             |
|                | fast  |                   | 7[]             |
|                | fast to very fast   | Morino Natsumi    | 8[]             |
|                | very fast   |                   | 9[]             |

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

|             | Characteristics                | Example Varieties | Note |
|-------------|--------------------------------|-------------------|------|
| 5.3<br>(10) | Cap: shape of vertical section |                   |      |
|             | umbilicate                     |                   | 1[]  |
|             | flat                           |                   | 2[]  |
|             | convex                         |                   | 3[]  |
|             | umbonate                       |                   | 4[]  |
| 5.4<br>(11) | Cap: diameter                  |                   |      |
|             | very small                     |                   | 1[ ] |
|             |                                |                   |      |
|             | very small to small            |                   | 2[]  |
|             | small                          | Morino Natsumi    | 3[]  |
|             | small to medium                |                   | 4[]  |
|             | medium                         | HS73              | 5[]  |
|             | medium to large                |                   | 6[]  |
|             | large                          |                   | 7[]  |
|             | large to very large            |                   | 8[]  |
|             | very large                     |                   | 9[]  |
| 5.5<br>(12) | Cap: color of upper side       |                   |      |
|             | white                          | Kirko 989         | 1[]  |
|             | yellowish brown                | HS73              | 2[]  |
|             | brown                          | Susono 360, HS607 | 3[]  |
|             | reddish brown                  | Akiyama A-526     | 4[]  |

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

|             | Characteristics  | Example Varieties                     | Note |
|-------------|--|---------------------------------------|------|
| 5.6<br>(13) | Cap: thickness   |                                       |      |
|             | very thin  |                                       | 1[]  |
|             | very thin to thin  |                                       | 2[]  |
|             | thin   | Morino Harumitsu                      | 3[]  |
|             | thin to medium   |                                       | 4[]  |
|             | medium   | Susono 360, Morno<br>Natsumi, KX-S055 | 5[]  |
|             | medium to thick  |                                       | 6[]  |
|             | thick  | Akiyama A-526§                        | 7[]  |
|             | thick to very thick  |                                       | 8[]  |
|             | very thick   |                                       | 9[]  |
| 5.7<br>(23) | Stipe: shape   |                                       |      |
|             | cylindrical  |                                       | 1[]  |
|             | thick to upper part  |                                       | 2[]  |
|             | thick to lower part  |                                       | 3[]  |
|             | UA: The points 5.8 in TQ are not very clear for our experts  |                                       |      |
| 5.8         | Zone line of colony: dual culture of mother variety          |                                       |      |
|             | absent   |                                       | 1[]  |
|             | present  |                                       | 9[]  |
| 5.9         | Zone line of colony: dual culture of father variety          |                                       |      |
|             | absent   |                                       | 1[]  |
|             | present  |                                       | 9[]  |
|             | UA: The points 5.10 in TQ are not very clear for our experts |                                       |      |
| 5.10        | Zone line of colony: dual culture of similar variety         |                                       |      |
|             | absent   |                                       | 1[]  |
|             | present  |                                       | 9[]  |

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

|              | Characteristics  | Example Varieties                                 | Note |
|--------------|--|---|------|
|              | UA: In TQ for characteristics 35 example stains are absent   |   |      |
| 5.11<br>(35) | Fruit body: period from inoculation to fruiting induction    |   |      |
|              | very short   |   | 1[]  |
|              | very short to short  |   | 2[]  |
|              | short  | HS73(S), Kinko 702(B),<br>A-555(B)                | 3[]  |
|              | short to medium  |   | 4[]  |
|              | medium   | HS608(B), S-035(B),<br>Kinko 697(B)               | 5[]  |
|              | medium to long   |   | 6[]  |
|              | long   | Mori-yujiro(B), HS705(S),<br>S-035(S), ML8(S)     | 7[]  |
|              | long to very long  |   | 8[]  |
|              | very long  |   | 9[]  |
|              | UA: In TQ for characteristics 36 example stains are absent   |   |      |
| 5.12<br>(36) | Fruit body: <u>period</u> from fruiting induction to harvest |   |      |
|              | very short   |   | 1[]  |
|              | very short to short  |   | 2[]  |
|              | short  | KX-S055(B),<br>A-555(B),<br>HS-73(S),<br>S-005(S) | 3[]  |
|              | short to medium  |   | 4[]  |
|              | medium   | ML8(B),<br>ML8(S),<br>S-035(S)                    | 5[]  |
|              | medium to long   |   | 6[]  |
|              | long   | ML8(B),<br>ML8(S),<br>S-035(S)                    | 7[]  |
|              | long to very long  |   | 8[]  |
|              | very long  |   | 9[]  |

| TECHNICAL QUEST   | IONNAIRE  | Page {x}          | of {y}                 | Reference N  | Jumber:  |  |  |  |
|---|---|-------------------|------------------------|--|--|--|--|--|
| <u></u>   |   |                   |                        |  |  |  |  |  |
| 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<br>your candidate variety   | Characterist<br>which your c<br>variety differs<br>similar vari | andidate from the | of the char<br>for the | e expression<br>racteristic(s)<br>similar<br>ty(ies) | Describe the expression<br>of the characteristic(s)<br>for your candidate<br>variety |  |  |  |
| Example   | To be pro   | vided             | To be p                | provided   | To be provided   |  |  |  |
|   |   |                   |                        |  |  |  |  |  |
|   |   |                   |                        |  |  |  |  |  |
| Comments:   | Comments:   |                   |                        |  |  |  |  |  |

| TEC             | CHNICAL QU    | JESTIONNAIRE   | Page {x} of {y}          | Reference Number:                  |  |  |  |
|-----------------|---------------|--|--------------------------|------------------------------------|--|--|--|
| <sup>#</sup> 7. | Additional    | information which  | may help in the examin   | nation of the variety              |  |  |  |
| 7.1             | In addition   | 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 [   | 1                        |                                    |  |  |  |
|                 | (If yes, plea | ase provide details)   |                          |                                    |  |  |  |
| 7.2             | Are there a   | ny special condition   | ns for growing the varie | ety or conducting the examination? |  |  |  |
|                 | Yes [ ]       | No [   | ]                        |                                    |  |  |  |
|                 | (If yes, plea | ase provide details)   |                          |                                    |  |  |  |
| 7.3             | Other infor   | mation   |                          |                                    |  |  |  |
|                 | 7.3.1 Mai     | in type of cultivation   | n                        |                                    |  |  |  |
|                 | (a)           | log cultivation  |                          | [ ]                                |  |  |  |
|                 | (b)           | sawdust cultivatio   | n                        | [ ]                                |  |  |  |
|                 |               |  |                          |                                    |  |  |  |
| 8.              | Authorizati   | ion for release  |                          |                                    |  |  |  |

Does the variety require prior authorization for release under legislation concerning

[ ]

[ ]

the protection of the environment, human and animal health?

Has such authorization been obtained?

No

No

If the answer to (b) is yes, please attach a copy of the authorization.

Yes

Yes

(b)

[ ]

[ ]

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

TG/SHIITK(proj.2) Shiitake, 2010-05-21 - 33 -

| TECHN                         | TECHNICAL QUESTIONNAIRE   Page {x} of {y}   Reference Number:   |                             |                          |         |        |        |  |
|-------------------------------|---|-----------------------------|--------------------------|---------|--------|--------|--|
|                               | •   |                             |                          |         |        |        |  |
| by factoreffects              | 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.  |                             |                          |         |        |        |  |
| express<br>request<br>treatme | 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 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: |                             |                          |         |        |        |  |
| (8                            | (a)   | Microorganisms (e.g. viru   | us, bacteria, phytoplasi | ma) Y   | es [ ] | No [ ] |  |
| (ł                            | (b)   | Chemical treatment (e.g.    | growth retardant, pesti  | cide) Y | es [ ] | No [ ] |  |
| (0                            | (c)   | Tissue culture              |                          | Y       | es [ ] | No [ ] |  |
| (0                            | (d)   | Other factors               |                          | Y       | es [ ] | No [ ] |  |
| P                             | Please  | e provide details for where | e you have indicated "   | yes".   |        |        |  |
|                               | •••••   |                             |                          |         |        |        |  |
|                               | 10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:   |                             |                          |         |        |        |  |
| A                             | Applic  | cant's name                 |                          |         |        |        |  |
| Si                            | Signature Date  |                             |                          |         |        |        |  |

[End of document]