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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

SHIITAKE

UPOV Code: LENTI_EDO

Lentinula edodes (Berk.) Pegler

[*]

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 fifth session, to be held in Monterey, California, United States of America,
from July 25 to 29, 2011

Alternative Names:[*]

Latin	*English*	*French*	*German*	*Spanish*
Lentinula edodes (Berk.) Pegler *Lentinus elodes* (Berk.) Sing.	Shiitake Oak Mushroom	Shiitake	Pasaniapilz	Shiitake

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.]

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1. Subject of these Test Guidelines

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

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of spawn and as a pure culture on a suitable medium.

(a) Spawn 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 should have been stored under proper conditions.

(b) Pure cultures must be on slant agar tubes with an appropriate medium such as PDA (potato dextrose agar) or malt extract agar. Tubes should be covered by cotton plugs or plastic caps allowing sterile air diffusion. Cultures should be fresh, i.e. not stored for longer than 2 weeks at low temperature.

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

2 liter of spawn and 3 slant tubes containing secondary mycelium by pure culture.
[see Additional information (b)]

2.4 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

The minimum duration of tests should normally be two independent growing cycles. The growing cycle is considered to be from spawning until the end of 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”.

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 Each test should be designed to result in a total of at least 50 bed-logs or 50 sawdust blocks, which should be divided between at least two replicates.
Kr: For variance analysis, test design should be divided between at least three 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.

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, for the purposes of distinctness, all observations on single fruit bodies should be made on 50 fruit bodies or parts taken from each of 50 fruit bodies and any other observations made on all fruit bodies in the test, disregarding any off-type fruit bodies.

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

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 50 fruit bodies, 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:

(a) Cultivation type (1: bed-log cultivation type, 2: sawdust cultivation type) (TQ)
(b) Cap: shape of vertical section (characteristic 10)
(c) Cap: main color of apex (characteristic 12)
(d) Cap: presence of scale (characteristic 15)
(e) Gill: presence of gill (characteristic 19)
(f) Stipe: shape of vertical section (characteristic 24)

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. The type is indicated in brackets after the name of the example variety as follows:

(B) bed-log cultivation type
(S) sawdust cultivation type

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

MG, MS, VG, VS – see Chapter 4.1.5

(a) – (c) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.2

(B) bed-log cultivation type
(S) sawdust cultivation type

”Cultivation type” is only necessary for example varieties provided for Char.36, 37

See Explanations of the growing types in Chapter 8.3

Highlight indicate the major amendment and opinions or suggestions etc.

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

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. **(+)**	**VG**	**Density of hyphae on the medium**					
QN	**(a)**	sparse				HS607, Mori XR1	1
		intermediate				Morino Natsumi	2
		dense				KX-S005	3
2. **(+)**	**VG**	**Aerial hyphae: development on the medium**					
QN	**(a)**	weak				HS607, Mori XR1	1
		intermediate				Kinko 115, ML8	2
		strong					3
3. **(+)**	**VG**	**Colony: tinting of surface on the medium**					
QL	**(a)**	absent				Kinko 115, Mori XR1, Morino Natsumi	1
		present				HS607, KX-S005	9
4. **(+)**	**MS**	**Mycelium: optimum temperature for growth**					
QN	**(b)**	Lower than 23°C					1
		23°C				Kinko 243	2
		25°C				HS607, Kinko 115	3
		27°C				Morino Natsumi	4
		higher than 27°C					5

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
5. **(+)**	**MS**	**Mycelium: growth rate at 10°C**					
QN	**(b)**	very slow				Kinko 115	1
		slow				Kinoh1	2
		medium				HS607, Morino Natsumi	3
		fast				KX-S005	4
		very fast				Yujiro	5
6. **(+)**	**MS**	**Mycelium: growth rate at 15°C**					
QN	**(b)**	very slow					1
		slow				Kinko 115	2
		medium				HS607, Susono 360	3
		fast				Yujiro	4
		very fast				KX-S005	5
7. **(*)** **(+)**	**MS**	**Mycelium: growth rate at 20°C**					
QN	**(b)**	very slow				Bridge 32, Kinno 1	1
		slow				Kinko 115	2
		medium				ML8, Morino Natsumi	3
		fast				Morino Harumitsu	4
		very fast				Akiyama A-950, Hokken 600, JMS 237	5

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
8. **(+)**	**MS**	**Mycelium: growth rate at 25°C**					
QN	**(b)**	very slow					1
		slow				Kinko 115	2
		medium				HS73, Susono 360	3
		fast				Hokken600, Yujiro	4
		very fast					5
9. **(*)** **(+)**	**MS**	**Mycelium: growth rate at 30°C**					
QN	**(b)**	very slow				Akiyama A-526	1
		slow				HS73, Morino Harumitsu	2
		medium				Kinko 115	3
		fast				Mori XR1, Susono 360	4
		very fast				Morino Natsumi, Yujiro	5
JP: propose to change the expression of the state from “umbilicate, convex, embonate” to “concave, round, convex”.							
10. **(*)** **(+)**	**VG**	**Cap: shape of vertical section**					
PQ	**(c)**	umbilicate=> concave				JMS 7H-1	1
		flat				Morino Harumitsu	2
		convex => round				Kinko 115, Yujiro	3
		umbonate => convex				KX-S005	4

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
JP: propose to change the expression of the state from “narrow, medium, broad” to “small, medium, large”.							
11. **(*)** **(+)**	**VG/ MS**	**Cap: diameter**					
QN	**(c)**	very narrow => very small				○○○	1
		narrow => small				Morino Harumitsu	3
		medium				HS73, Kinko 115, Mori XR1	5
		broad => large				Kinko 117, Mori 505	7
		very broad => very large				○○○	9
12. **(*)**	**VG**	**Cap: main color of apex**					
PQ	**(c)**	white				Kinko 989	1
		yellowish brown				Mori XR-1	2
		brown				Kinko 115, Susono 360	3
		reddish brown				Akiyama A-526	4
13. **(+)**	**MS**	**Cap: height**					
QN	**(c)**	low				Morino Harumitsu	3
		medium				Mori XR1, Morino Natsumi, Susono 360	5
		high				Akiyama A-526	7
14. **(+)**	**VS**	**Cap: firmness**					
QN	**(c)**	soft				Kinko 650	1
		intermediate				HS607, Kinko 115, KX-S055, Susono 360	2
		solid				Morino Natsumi	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
15. (*) (+)	**VG**	**Cap: presence of scale**					
QL	**(c)**	absent				KX-S034	1
		present				HS73, Kinko 115, ML8, Morino Natsumi	9
16. (+)	**VG**	**Cap: distribution of scale**					
QN	**(c)**	whole				Kinko 115, Mori XR1	1
		periphery				Morino Natsumi, Susono 360, Yujiro	2
17. (+)	**VG**	**Cap: size of scale**					
QN	**(c)**	small				HS73, Mori XR1	3
		medium				Morino Natsumi, Susono 360, Yujiro	5
		large				Kinko 169	7
JP: propose to change example variety							
18. (+)	**VG**	**Cap: tinting of scale**					
QL	**(c)**	absent				~~Kinko 115~~ => JMS5K16, ML8, Morino Natsumi	1
		present				HS73, Yujiro	9
19. (*) (+)	**VG**	**Cap: presence of gill**					
QL	**(c)**	absent				FERM P-14310	1
		present				Kinko 115, Mori XR1	9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
JP: Propose to delete from the asterisked characteristics. These states might be different the inside and outside of curved stipe. It is necessary to be observed the gill located outside of curved stipe. The evaluation of the note 2 is very difficult. Delite note 2.							
20. ~~**(*)**~~ **(+)**	**VG**	**Gill: shape**					
QL ?	**(c)**	separate from stipe				Kinko 115, Yujiro	1
		~~partly attached to stipe~~ ?				~~Morino Natsumi, Mori XR1~~ ?	~~2~~ ?
		~~fully~~ attached to stipe				Hokken 600, Mori 505	3
21. **(*)** **(+)**	**VG**	**Gill: arrangement of row**					
QL	**(c)**	straight				Kinko 115, KX-S055, Morino Natsumi	1
		ripple or crinkle				Akiyama A-526, Mori XR1	2
22. **(+)**	**VG**	**Gill: width**					
QN	**(c)**	very narrow				Mori XR1	1
		narrow				Yujiro	3
		medium				Susono 360	5
		wide				KX-S034	7
		very wide					9
23. **(+)**	**VG**	**Gill: density of row**					
QN	**(c)**	sparse				Kinko 169, Mori 476	1
		medium				Yujiro	2
		dense				Kinko 115, Mori XR1, Morino Natsumi	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24.	**VG**	**Gill: color**					
PQ	**(c)**	white				Kinko 115, Mori XR1, Morino Natsumi	1
		light yellow				HS607, KX-S055	2
		light yellow orange				HS73	4
JP: propose to add example variety							
25. **(*)** **(+)**	**VG**	**Stipe: shape in vertical section**					
PQ	**(c)**	broader toward cap				Susono 360	1
		cylindrical				JMS5K16, Kinko 115, Mori XR1, Morino Natsumi	2
		broader toward base				JMS 7H-1	3
26. **(*)** **(+)**	**MS**	**Stipe: length**					
QN	**(c)**	very short				○○○	1
		short				Mori XR1, Morino Natsumi, Susono 360	3
		medium				HS702, Kinko 117	5
		long				Akiyama A-526	7
		very long				○○○	9
JP: Propose to change the expression of the state from “narrow, medium, broad” to “small, medium, large”.							
27. **(+)**	**MS**	**Stipe: diameter**					
QN	**(c)**	narrow => small				Morino Natsumi	3
		medium				HS73, Susono 360	5
		broad => large				Kinko 115	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
28. (*) (+)	**VG**	**Stipe: tinting**					
QL	**(c)**	absent				Mori XR1	1
		present				Kinko 115, KX-S055 Morino Natsumi	9
29. (+)	**VG**	**Stipe: density of fluff**					
QN	**(c)**	absent or sparse				Kinko 989	1
		medium				Kinko 115, KX-S055, Morino Natsumi	2
		dense				KB-2010	3
30. (+)	**VG**	**Stipe: tinting of fluff**					
QL	**(c)**	absent				KX-S055, Mori XR1	1
		poresent				Kinko 115, Morino Natsumi	9
31. (+)	**VG**	**Stipe: firmness**					
QN	**(c)**	soft				HS802, Kinno 7	1
		intermediate				HS607, Mori XR1, Susono 360	2
		solid				Kinko 115	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
32. **(+)**	**MS**	**Fruit body: ratio of cap diameter / stipe length**					
QN	**(c)**	cap far shorter than stipe length				Kinko 610	1
		cap shorter than stipe length				Mori 252	3
		cap almost equal to stipe length				Akiyama A-526, Susono 360	5
		cap longer than stipe length				Morino Natsumi	7
		cap far longer than stipe length				Morino Harumitsu	9
33.	**MS**	**Fruit body: ratio of cap diameter / stipe diameter**					
QN	**(c)**	cap far shorter than stipe diameter					1
		cap shorter than cap diameter				ML12	3
		cap almost equal to cap diameter				JHS KV92	5
		cap longer than stipe diameter				Morino Harumitsu	7
		cap far longer than stipe diameter				Mori 4T98	9
34. **(+)**	**MG**	**Fruit body: dry weight at harvest maturity**					
QN	**(c)**	light				HS73	3
		medium				Akiyama A-526, Susono 360, Yujiro	5
		heavy					7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
35.	**MS**	**Fruit body: ratio of oven-dry weight / fresh weight**					
QN	**(c)**	oven-dry weight far lighter than fresh weight				KX-S005, Morino Natsumi, Susono 360	1
		oven-dry weight lighter than fresh weight				HS73, Kinko 115, Yujiro	2
		oven-dry weight little lighter than fresh weight				HS607, JMS 888, Morino Harumitsu	3
~~**35. (+)**~~	~~**VG**~~	~~**Fruit body: type of fruiting**~~	**HU: 35 and 37 is almost the same. We propose to keep 36. and delete 34. If 34 will be kept, explanation is necessary.** **JP: delete. In the duration from the beginning of the fruiting to the end, there are aggregated type and scattered type. But the evaluation and definition as the Char. of QL is difficult.**				
~~PQ~~	~~**(c)**~~	~~aggregate~~				~~Susono 360, Morino Natsumi, KX-S055~~	~~1~~
		~~scattering~~				~~Yujiro, ML8~~	~~2~~
36. (*) (+)	**VG**	**Fruit body: period from inoculation to fruiting treatment**					
QN		short				A-555(B), HS73(S), Kinko 702(B)	3
		medium				HS608(B), Kinko 697(B), S-035(B),	5
		long				HS705(S), Kinko 169(B), ML8(S) Mori-yujiro(B), S-035(S)	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
37. (*) (+)	**VG**	**Fruit body: period from fruiting treatment to harvest**					
QN		short				A-555(B), HS-73(S), KX-S055(B), S-005(S)	3
		medium				A-526(B), HS72(S), HS705(S)	5
		long				ML8(B), ML8(S), S-035(S)	7

8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

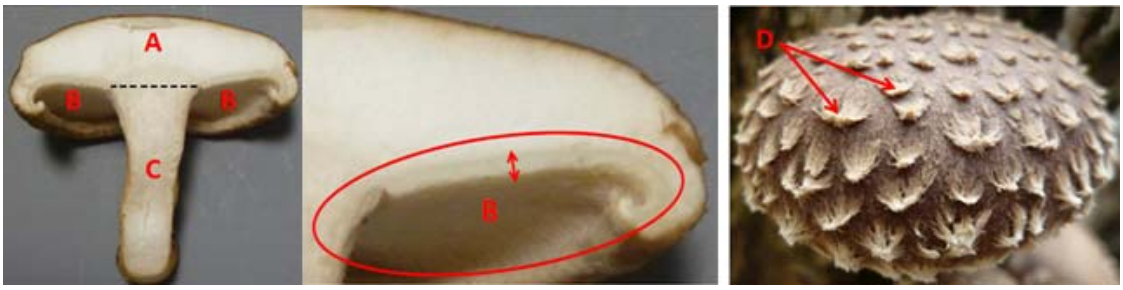
Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) Hyphae and Colony: should be observed by pure cultures.
Type of medium : PDA (Potato dextrose agar)
Plate : 9cm in inside diameter and 2cm in height
Conditions : in the dark at 25 ± 2°C
Observations : (hyphae) developed to about 70% of the diameter of the plate
(colony) 14 days after
Number of plate : more than two

(b) Mycelium: 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
Number of tube : more than five

(c) Stipe, cap and gills: Unless otherwise indicated, all characteristics of the fruit bodies (the cap, the stipe and the gills) should be recorded at 80 to 90% open gills (stage 4[see Additional information (a)] handpicked mushrooms; freshly harvested).

(d) General illustration:



A: cap B: gill C: stipe D: scale

8.2 Explanations for individual characteristics

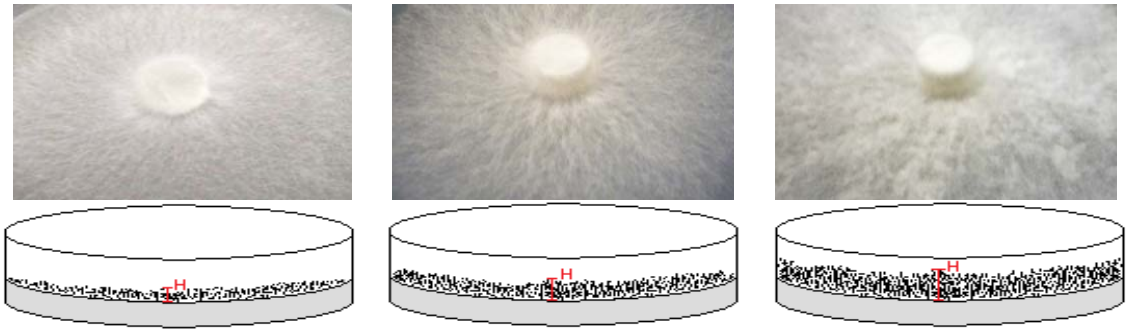
Ad. 1: Density of hyphae on the medium

Observe the dense of hyphae of the PDA medium (see 8.1 (b)).

1 sparse | 2 intermediate | 3 dense

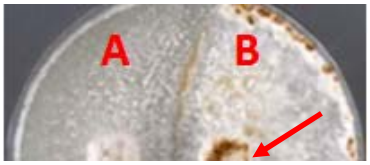
Ad. 2: Aerial hyphae: development on the medium

Observe the height from the surface of PDA medium to the top of aerial hyhae (see 8.1 (b)).



1	2	3
weak	intermediate	strong

Ad. 3: Colony: tinting of surface on the medium

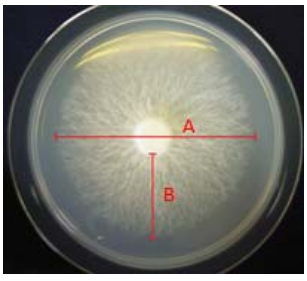


Observe the presence of tinting of colony after 14 days cultivation (see 8.1 (b)).

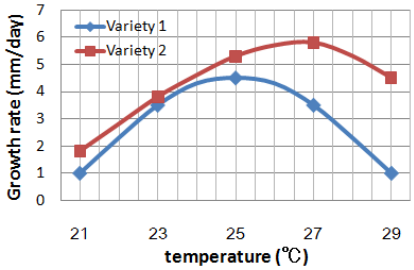
A: 1 absent
B: 9 present

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

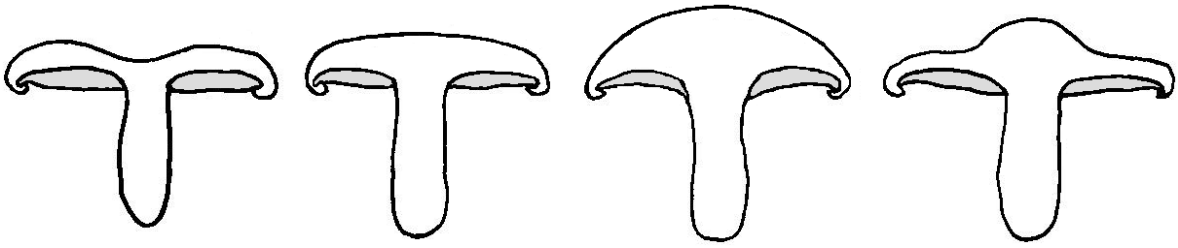
Measure the length or diameter (see 8.1(b) tube/plate) that grew up on the 14th day from the 4th day of mycelium cultured at each temperature. The incubation temperature of mycelium that grows up the longest becomes an optimum temperature. Amount of mycelium growth per day at each temperature are considered to be the growth rate. These characteristics should be evaluated by drawing of mycelium growth curve (see following graph).



A: diameter of mycelium
B: length of ~~mycelium~~
⇨ colony



Ad. 10: Cap: shape of vertical section



1
~~umbilicate~~
concave

2
flat

3
~~convex~~
round

4
~~umbonate~~
convex

Ad. 11: Cap: diameter
Ad. 13: Cap: height
Ad. 22: Gill: width

Measure the widest part of the gill.

Ad. 14: Cap: firmness

Determine by hand the hardness of the cap is compared to standard varieties

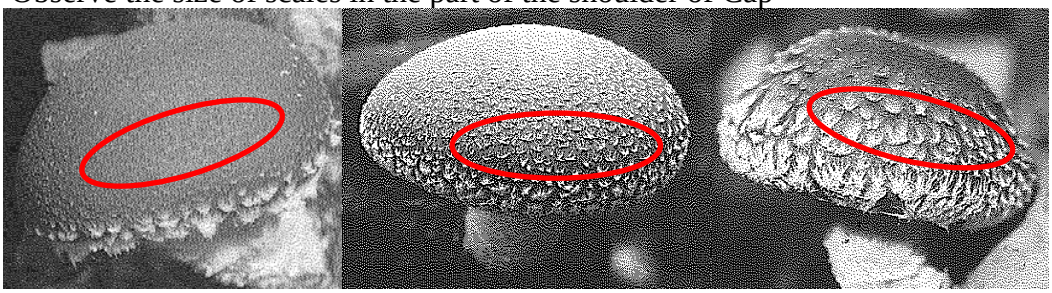
Ad. 16: Cap: distribution of scale

1
whole

2
periphery

Ad. 17: Cap: size of scale

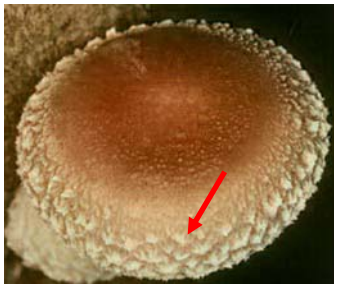
Observe the size of scales in the part of the shoulder of Cap



3	5	7
small	medium	large

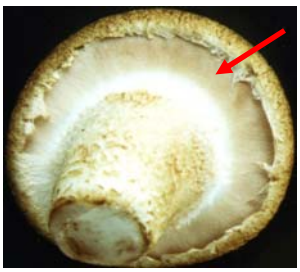
Ad. 18: Cap: tinting of scale

1
absent



9
present

Ad. 19: Cap: presence of gill

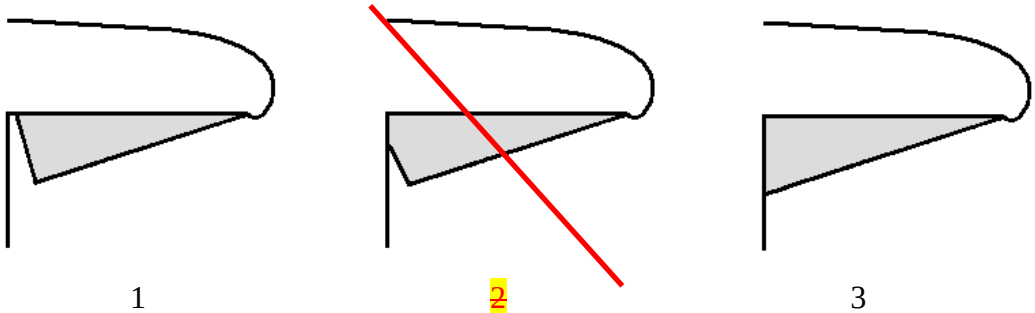


1
absent

9
present

Ad. 20: Gill: shape

Observation should be made on the gill located outside of curved stipe.



1
separate from stipe

~~2~~
~~partly attached to stipe~~

3
~~fully~~ attached to stipe

Ad. 21: Gill: arrangement of row

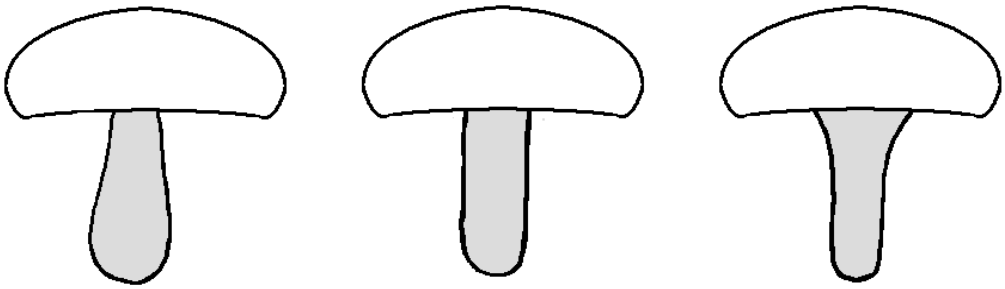
1
straight

2
ripple or crinkle

Ad. 23: Gill: density of row

1	2	3
sparse	medium	dense

Ad. 25: Stipe: shape in vertical section

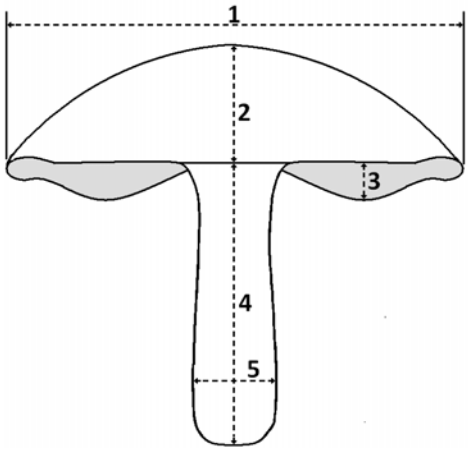


1	2	3
broader toward cap	cylindrical	broader toward base

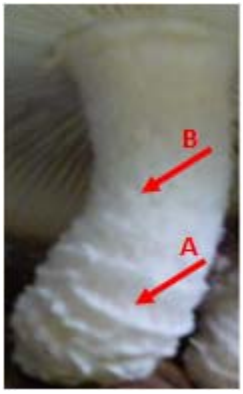
Ad. 26: Stipe: length
Ad. 27: Stipe: diameter

Measure the widest part of the stipe.

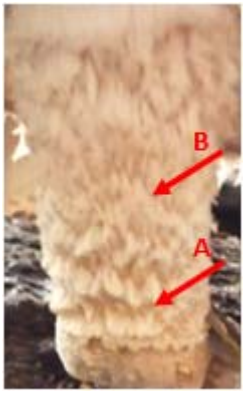
1 Cap: diameter
2 Cap: height
3 Gill: width
4 Stipe: length
5 Stipe: diameter



Ad. 28: Stipe: tinting
Ad. 30: Stipe: tinting of fluff



1
absent



2
present

A: Stipe surface
B: Fluff of stype

Ad. 29: Stipe: density of fluff

1
absent or sparse

2
medium

3
dense

Ad. 31: Stipe: firmness

Determine by hand the hardness of the stipe is compared to standard varieties.

Ad. 34: Fruit body: dry weight of harvest maturity

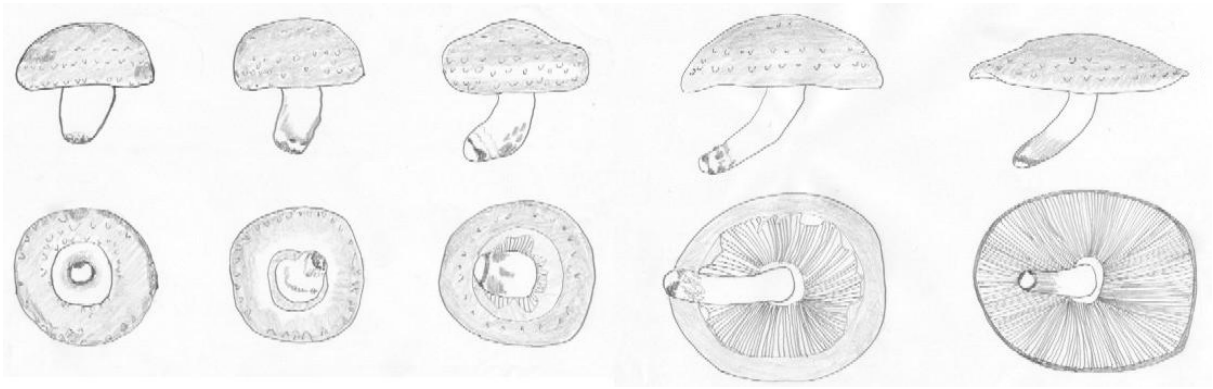
The fruiting body dries at 60℃ until becoming a constant weight.

Ad. 36: Fruit body: Period from inoculation to fruiting treatment
Ad. 37: Fruit body: Period from fruiting treatment to harvest

The fruiting treatment is indispensable for the fruit body development. In the same condition, the timing of the fruiting treatment is different according to each variety clearly. In the fruiting treatment, there is water soaking treatment, water sprinkling treatment, low temperature treatment, or physical treatment etc. Any method is stimulation for the fruiting body formation. In each cultivation type (bed-log cultivation, sawdust cultivation), the fruiting treatment should be treated at the time when the symptom (e.g. appearance of primordium) of the fruiting body formation is shown. The harvesting time is considered to be a peak from which fruit bodies are harvested most.

Additional information:

(a) Stage of fruit body

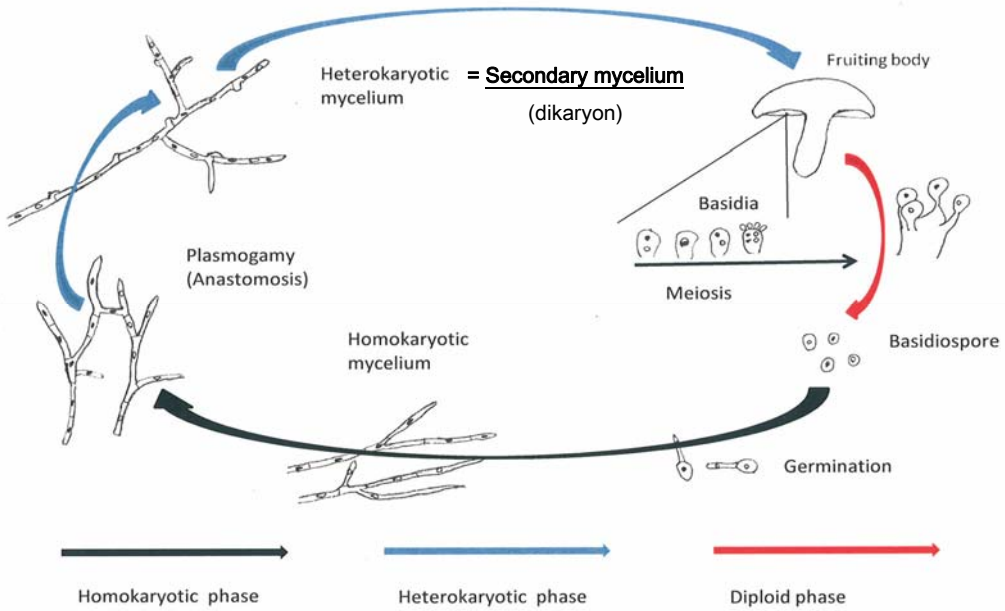


1 2 3 4 5

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

stage 4

(b) Life cycle of *Lentinula edodes* (Berk.) Pegler



8.3 Growing Types

As explained in Chapter 3.3.1, it may be necessary for separate growing trials to be established for bed-log cultivation types and sawdust cultivation types in order to ensure the satisfactory growth of varieties of those types. The following information is provided with regard to growing conditions for different types of varieties and information which may help in deciding on the type of trial(s) which may be appropriate for a variety:

Bed-log cultivation types

Breeding is done in a rather large gene pool, in most cases much broader than the sawdust cultivation types. In general, such types of variety have the following features:

- some varieties of this types don't develop the fruit bodies in the condition of the sawdust cultivation;
- mainly used as dried shiitake mushrooms and produced in the bed-log laying yard;
- the cultivation period is very long than the sawdust cultivation types;
- firmness of cap is comparatively hard;

Sawdust cultivation types

Breeding is done in a limited gene pool. In general, such types of variety have the following features:

- some varieties of this types don't develop the fruit bodies in the condition of the bed-log cultivation;
- tolerant of higher temperatures in general;
- mainly used as fresh shiitake mushrooms ;
- concern only types produced in sheltered conditions;
- the cultivation period is comparatively short;
- firmness of cap is comparatively soft;

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. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (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	*Lentinula edodes* (Berk.) Pegler
1.2 Common Name	Shiitake

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)

(……..……..…)	x	(……..……..…)
female parent		male parent

(b) partially known cross [ ]
(please state known parent variety(ies))

(……..……..…)	x	(……..……..…)
female parent		male parent

(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)”

4.2 Method of propagating the variety

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
5.1	**Growth type**		
	bed-log cultivation type	Morino Natsumi, Yujiro	1[ ]
	sawdust cultivation type	Hokken 600, Mori XR1	2[ ]
5.2 (7)	**Mycelium: growth at 20°C**		
	very slow	Bridge 32, Kinno 1	1[ ]
	slow	Kinko 115	2[ ]
	medium	ML8, Morino Natsumi	3[ ]
	fast	Morino Harumitsu	4[ ]
	very fast	Akiyama A-950, Hokken 600, JMS 237	5[ ]
5.3 (9)	**Mycelium: growth at 30°C**		
	very slow	Akiyama A-526	1[ ]
	slow	HS73, Morino Harumitsu	2[ ]
	medium	Kinko 115	3[ ]
	fast	Mori XR1, Susono 360	4[ ]
	very fast	Morino Natsumi, Yujiro	5[ ]
5.4 (10)	**Cap: shape of vertical section**		
	~~umbilicate~~ => concave	JMS 7H-1	1[ ]
	flat	Morino Harumitsu	2[ ]
	~~convex~~ => round	Kinko 115, Yujiro	3[ ]
	~~umbonate~~ => convex	KX-S005	4[ ]

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

	Characteristics	Example Varieties	Note
5.5 (11)	**Cap: diameter**		
	very narrow => small => very small	○○○	1[ ]
	very narrow to narrow => very small to small	○○○	2[ ]
	narrow => small	Morino Harumitsu	3[ ]
	narrow to medium => small ・・・	Morino Natsumi, Yujiro	4[ ]
	medium	HS73, Kinko 115, Mori XR1	5[ ]
	medium to broad => ・・・large	Akiyama A-526, HS607,	6[ ]
	broad=> large	Kinko 117, Mori 505	7[ ]
	broad to very broad => large to very large	Kinko 245, Kinko 130	8[ ]
	very broad=> very large	○○○	9[ ]
5.6 (12)	**Cap: main color of apex**		
	white	Kinko 989	1[ ]
	yellowish brown	Mori XR-1	2[ ]
	brown	Kinko 115, Susono 360	3[ ]
	reddish brown	Akiyama A-526	4[ ]
5.7 (15)	**Cap: presence of scale**		
	absent	KX-S034	1[ ]
	present	HS73, Kinko 115, ML8, Morino Natsumi	9[ ]
5.8 (19)	**Cap: presence of gill**		
	absent	FERM P-14310	1[ ]
	present	Kinko 115, Mori XR1	9[ ]

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

	Characteristics	Example Varieties	Note
5.9 (25)	**Stipe: shape in vertical section**		
	broader toward cap	Susono 360	1[ ]
	cylindrical	JMS5K16, Kinko 115, Mori XR1, Morino Natsumi	2[ ]
	broader toward base	JMS 7H-1	3[ ]
5.10 (26)	**Stipe: length**		
	broader toward cap	Susono 360	1[ ]
	cylindrical	Kinko 115, Mori XR1, Morino Natsumi	2[ ]
	very short	○○○	1[ ]
	very short to short	Yujiro, Morino Harumitsu	2[ ]
	short	Mori XR1,Morino Natsumi, Susono 360	3[ ]
	short to medium	Kinko 115	4[ ]
	medium	HS702, Kinko 117	5[ ]
	medium to long	HS73, KX-S005, HS607	6[ ]
	long	Akiyama A-526	7[ ]
	long to very long	HS802	8[ ]
	very long	○○○	9[ ]
5.11 (28)	**Stipe: tinting**	Mori XR1	1[ ]
	absent	Mori XR1	1[ ]
	present	Kinko 115, KX-S055 Morino Natsumi	9[ ]
5.12	**UA: The points 5.12 in TQ are not very clear for our experts** **JP: This characteristic is clear in the examination of the current Japan. The experts in Japan have the opinion that these characteristics are necessary in TQ.**	Kinko 115, Morino Natsumi, KX-S055	9[ ]
	Zone line of colony: dual culture of mother variety		
	absent		1[ ]
	present	Mori XR1	9[ ]

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

	Characteristics	Example Varieties	Note
5.13	**Zone line of colony: dual culture of father variety**		
	absent		1[ ]
	present	Mori XR1	9[ ]
5.14	**UA: The points 5.14 in TQ are not very clear for our experts** **JP: This characteristic is clear in the examination of the current Japan. The experts in Japan have the opinion that these characteristics are necessary in TQ.** **Zone line of colony: dual culture of similar variety**		
	absent		1[ ]
	present	Mori XR1	9[ ]
5.15 (36)	**Fruit body: period from inoculation to fruiting treatment**		
	very short	○○○	1[ ]
	very short to short		2[ ]
	short	HS73(S), Kinko 702(B), A-555(B)	3[ ]
	short to medium		4[ ]
	medium	HS608(B), S-035(B), Kinko 697(B)	5[ ]
	medium to long		6[ ]
	long	Mori-yujiro(B), HS705(S), S-035(S), ML8(S)	7[ ]
	long to very long		8[ ]
	very long	○○○	9[ ]

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

	Characteristics	Example Varieties	Note
5.16 (37)	**Fruit body: period from fruiting treatment to harvest**		
	very short	○○○	1[ ]
	very short to short		2[ ]
	short	KX-S055(B), A-555(B), HS-73(S), S-005(S)	3[ ]
	short to medium		4[ ]
	medium	A-526(B), HS72(S), HS705(S)	5[ ]
	medium to long		6[ ]
	long	ML8(B), ML8(S), S-035(S)	7[ ]
	long to very long		8[ ]
	very long	○○○	9[ ]

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: shape of vertical section*	*convex*	*flat*

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

7.3.1 Type of cultivation

(a) bed-log cultivation [ ]

(b) sawdust cultivation [ ]

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.

[#] 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:

9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a) Microorganisms (e.g. virus, bacteria, phytoplasma) Yes [ ] No [ ]

(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]