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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

ZOYSIA GRASSES

UPOV Code(s): ZOYSI

Zoysia Willd.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

*prepared by experts from Japan
 to be considered by the
 Technical Working Party for Agricultural Crops
 at its fiftieth session, to be held in Arusha, United Republic of Tanzania,
 from 2021-06-21 to 2021-06-25*

Disclaimer: this document does not represent UPOV policies or guidance

Alternative names:*

<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
<i>Zoysia</i> Willd.	Japanese Lawn Grass	Zoysia	Zoysia	Zoysia

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 *Zoysia* Willd.

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 plants or seeds.

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

vegetatively propagated varieties: 40 plants
seed-propagated varieties: 500 g of seed.

In the case of seed, the seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority.

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

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 *Number of Growing Cycles*

3.1.1 The minimum duration of tests should normally be two independent growing cycles.

3.1.2 The two independent growing cycles should be in the form of two separate plantings.

3.1.3 The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

3.2 *Testing Place*

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness".

3.3 *Conditions for Conducting the Examination*

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 In the case of vegetatively propagated varieties, each test should be designed to result in a total of at least 15 plants which should be divided between at least 3 replicates.

3.4.2 In the case of seed-propagated varieties, each test should be designed to result in a total of at least 60 which should be divided between at least 3 replicates.

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

3.5 *Additional Tests*

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 *Distinctness*

4.1.1 General Recommendations

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

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.1.4 Number of Plants or Parts of Plants to be Examined

In the case of vegetatively propagated varieties, unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts taken from each of 10 plants and any other observation made on all plants in the test, disregarding any off-type plants.

In the case of seed-propagated varieties, unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observation made on all plants in the test, disregarding any off-type plants.

4.1.5 Method of Observation

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

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

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

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

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

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

"Visual" observation (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 These Test Guidelines have been developed for the examination of vegetatively propagated varieties. For varieties with other types of propagation, the recommendations in the General Introduction and document TGP/13 "Guidance for new types and species" Section 4.5 "Testing Uniformity" should be followed.
- 4.2.3 The assessment of uniformity for seed-propagated varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.
- 4.2.4 For the assessment of uniformity of vegetatively propagated varieties, a population standard of 95% and an acceptance probability of at least 1% should be applied. In the case of a sample size of 15 plants, 1 off-type is allowed.

4.3 *Stability*

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

5. Grouping of Varieties and Organization of the Growing Trial

- 5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.
- 5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.
- 5.3 The following have been agreed as useful grouping characteristics:
- (a) Stolon: color (characteristic 9)
 - (b) Leaf: leaf blade length (characteristic 10)
 - (c) Leaf: shape of ligule (characteristic 17)
 - (d) Inflorescence: color of spikelets (characteristic 27)
 - (e) Time of flowering (in spring) (characteristic 31)
 - (f) Time of senescence of leaves (in autumn) (characteristic 32)
- 5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 "Examining Distinctness".

6. Introduction to the Table of Characteristics

6.1 *Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

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

6.1.2 Asterisked Characteristics

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

6.2 *States of Expression and Corresponding Notes*

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

6.2.2 All relevant states of expression are presented in the characteristic.

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

6.3 *Types of Expression*

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

6.4 *Example Varieties*

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

6.5 Legend

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

- 1 Characteristic number
- 2 (*) Asterisked characteristic – see Chapter 6.1.2
- 3 Type of expression
 QL Qualitative characteristic – see Chapter 6.3
 QN Quantitative characteristic – see Chapter 6.3
 PQ Pseudo-qualitative characteristic – see Chapter 6.3
- 4 Method of observation (and type of plot, if applicable)
 MG, MS, VG, VS – see Chapter 4.1.5
- 5 (+) See Explanations on the Table of Characteristics in Chapter 8.2
- 6 (a)-(d) See Explanations on the Table of Characteristics in Chapter 8.1
- 7 Not applicable

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

	English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.	QN	VG	(+)	(a)				
	Plant: growth habit							
		erect						1
		semi-erect					Meyer	3
		intermediate					Emerald	5
		semi-prostrate						7
		prostrate					Tsukuba hime	9
2. (*)	QN	MS/VG	(+)	(c)				
	Plant: height							
		short						3
		medium						5
		tall						7
3. (*)	QN	MS/VG	(+)					
	Stolon: length							
		narrow						3
		medium						5
		broad						7
4. (*)	QN	VG		(a)				
	Stolon: anthocyanin coloration of leaf sheath							
		absent or very weak						1
		weak						3
		medium						5
		strong						7
		very strong						9
5.	QN	MS		(a)				
	Stolon: length of leaf sheath							
		short						3
		medium						5
		long						7

	English		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
6.	QN	VG	(a)				
	Stolon: density of hairiness on leaf sheath						
	sparse						1
	medium						3
	dense						5
7. (*)	QN	MS	(+)	(a)			
	Stolon: internode length						
	short						3
	medium						5
	long						7
8.	QN	MS	(+)	(a)			
	Stolon: width of internode						
	narrow						1
	medium						3
	broad						5
9. (*)	PQ	VG		(a)			
	Stolon: color						
	greenish yellow						1
	light green						2
	medium green						3
	dark green						4
	reddish green						5
	red						6
	purple						7
10 (*)	QN	MS/VG		(a), (d)			
	Leaf: leaf blade length						
	short					Emerald	3
	medium						5
	long						7

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
11	(*) QN MS/VG	(a), (d)				
	Leaf: leaf blade width					
	narrow					3
	medium					5
	broad					7
12	QN VG	(a)				
	Leaf: leaf blade intensity of green color					
	light					3
	medium				Emerald	5
	dark				Meyer	7
13	(*) QN VG	(a)				
	Leaf: leaf blade density of hairs on upper side					
	absent or very sparse					1
	sparse					2
	medium					3
	dense					4
	very dense					5
14	QN VG	(a)				
	Leaf: leaf blade density of hairs on lower side					
	absent or very sparse					1
	sparse					3
	medium					5
	dense					7
15	QL VG	(a)				
	Leaf: leaf blade margin					
	smooth					1
	serrated					2

	English		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
16	QN	VG	(a)				
	Leaf: leaf sheath density of hairs						
	sparse						1
	medium						3
	dense						5
17 (*)	PQ	VS	(+)	(a)			
	Leaf: shape of ligule						
	absent						1
	membranous						2
	fringe of hairs						3
18	QN	MS	(b)				
	Culm: length						
	short						1
	medium						3
	long						5
19	QN	MS	(b)				
	Culm: width						
	narrow						3
	medium						5
	broad						7
20	QN	MS/VG	(b)				
	Flag leaf: length						
	short						3
	medium						5
	long						7
21	QN	MS/VG	(b)				
	Flag leaf: width						
	narrow						3
	medium						5
	broad						7

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
22 (*)	QN MS/VG	(b)				
	Inflorescence: length					
	short					3
	medium					5
	long					7
23	QN MS/VG	(b)				
	Inflorescence: width					
	narrow					3
	medium				Meyer	5
	broad					7
24 (*)	QN VG					
	Plant: number of inflorescences (in spring)					
	none or very few					1
	few					3
	medium					5
	many				Meyer	7
	very many					9
25	QN VG					
	Plant: number of inflorescences (in autumn)					
	none or very few					1
	few					3
	medium					5
	many					7
	very many					9
26	QN VG	(+)				
	Inflorescence: position of inflorescence from vegetative growth area					
	below				GZ-006	1
	medium				G-10	2
	above				Diamond	3

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
27 (*)	PQ VG					
	Inflorescence: color of spikelets					
	yellowish					1
	greenish					2
	purplish				Emerald, Meyer	3
28 (*)	QN MS/VG					
	Inflorescence: length of spikelets					
	short					3
	medium				Meyer	5
	long					7
29	QN MS/VG					
	Inflorescence: number of spikelets					
	few					3
	medium					5
	many					7
30	QN MG	(+)				
	Time of appearance of new leaves					
	early					3
	medium					5
	late					7
31 (*)	QN MG	(+)				
	Time of flowering (in spring)					
	early					3
	medium				Emerald, Meyer	5
	late					7
32 (*)	QN MG	(+)				
	Time of senescence of leaves (in autumn)					
	early					3
	medium				Emerald	5
	late					7

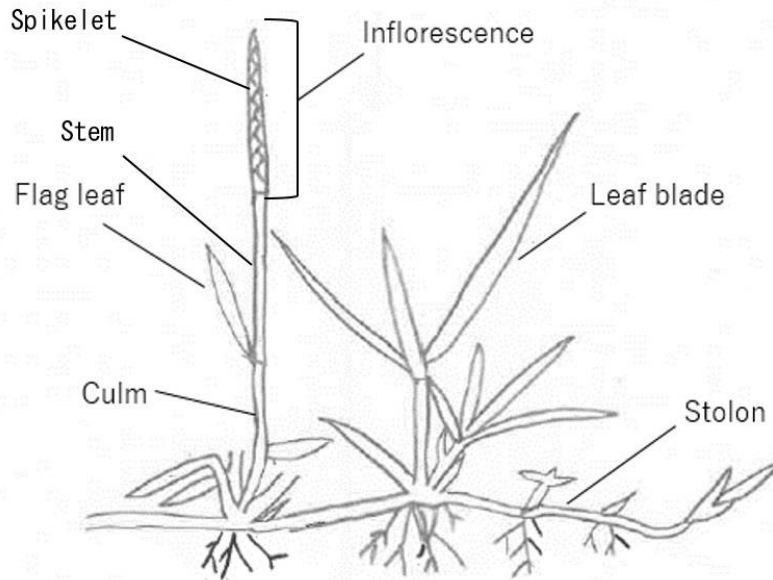
	English		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
33	QN	VG					
	Colored leaves: intensity of anthocyanin coloration (in autumn)						
	absent or very weak						1
	weak						3
	medium						5
	strong						7
	very strong						9

8. Explanations on the Table of Characteristics

8.1 *Explanations covering several characteristics*

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

- (a) The plant, stolon and leaf should be observed just before flowering time
- (b) The culm, flag leaf and inflorescence should be observed at flowering time
- (c) Explanation on the plant parts of Japanese lawn grass.

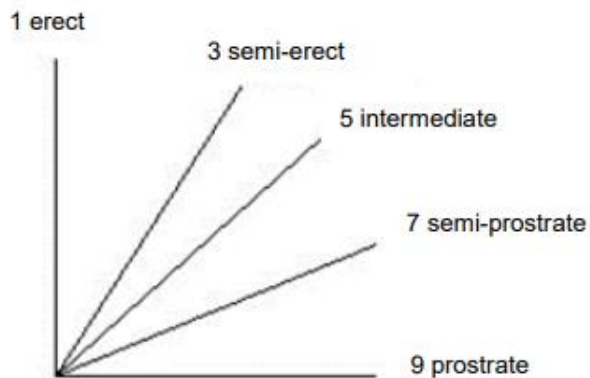


- (d) Observation on the leaf blade should be made on the largest leaf of the upright stem in the middle of the plant.

8.2 *Explanations for individual characteristics*

Ad. 1: Plant: growth habit

Plant growth habit is assessed at the vegetative stage just before flowering or during the early flowering stage. It should be assessed visually from the attitude of the leaves and the development of lateral stolons. The angle formed by the outer leaves with an imaginary middle axis should be used. The following 1-9 scale is used to describe the states.



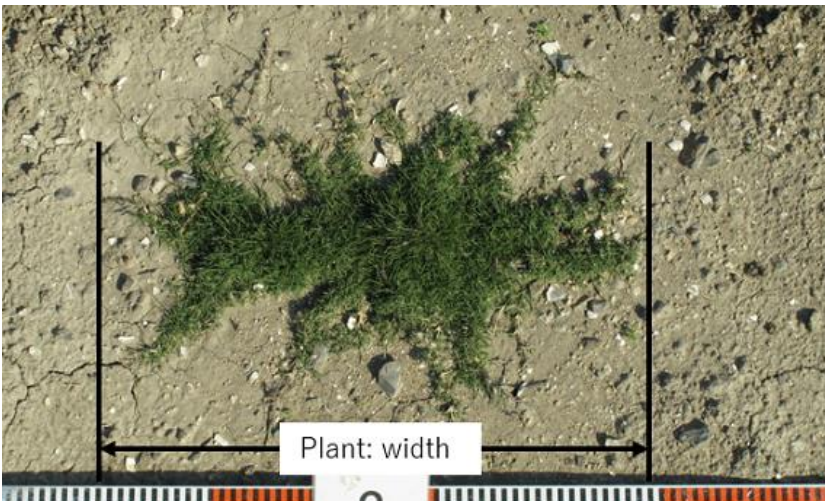
Ad. 2: Plant: height

Plant height should be observed in the summer of the 2nd year.



Ad. 3: Stolon: length

Stolon length should be observed in the summer in the 2nd year.



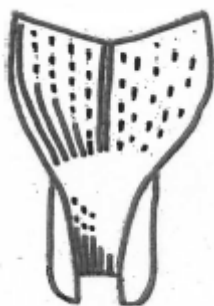
Ad. 7: Stolon: internode length

Internode length of stolon should be observed between 4 and 5 nodes from tip of stolon.

Ad. 8: Stolon: width of internode

Internode width of stolon should be observed between 4 and 5 nodes from tip of stolon.

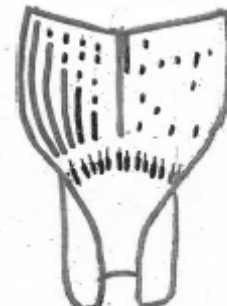
Ad. 17: Leaf: shape of ligule



1
absent



2
membranous



3
fringe of hairs

Ad. 26: Inflorescence: position of inflorescence from vegetative growth area



1
below

2
medium



3
above

Ad. 30: Time of appearance of new leaves

Time of greening should be observed the day when new leaves can be seen on the stems of about 50% of the plants after vernalization.

Ad. 31: Time of flowering (in spring)

Time of flowering in spring should be observed in the 2nd year after planting.

Ad. 32: Time of senescence of leaves (in autumn)

Time of coloring should be observed the day when the leaves color changed about its of the leaves 50% of the plant from autumn to early winter.

9. Literature

Japanese Society of Turfgrass Science., 2001: Handbook: management of turf and turfgrass research. Soft science Co., Tokyo, Japan

Asano, T., Aoki, K., 1998: Turfgrasses and the cultivars., Soft science Co., Tokyo, Japan

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	<input type="text" value="Zoysia Willd."/>
1.2	Common name	<input type="text" value="Japanese Lawn Grass"/>
2. Applicant		
	Name	<input type="text"/>
	Address	<input type="text"/>
	Telephone No.	<input type="text"/>
	Fax No.	<input type="text"/>
	E-mail address	<input type="text"/>
	Breeder (if different from applicant)	<input type="text"/>
3. Proposed denomination and breeder's reference		
	Proposed denomination (if available)	<input type="text"/>
	Breeder's reference	<input type="text"/>

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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#4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross

(please state parent variety)

(.....) 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)

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:
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4.2 Method of propagating the variety	[]
4.2.1 Other (Please provide details)	
<input type="text"/>	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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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 Stolon: color (9)		
greenish yellow		1 []
light green		2 []
medium green		3 []
dark green		4 []
reddish green		5 []
red		6 []
purple		7 []
5.2 Leaf: leaf blade length (10)		
short	Emerald	3 []
medium		5 []
long		7 []
5.3 Leaf: shape of ligule (17)		
absent		1 []
membranous		2 []
fringe of hairs		3 []
5.4 Inflorescence: color of spikelets (27)		
yellowish		1 []
greenish		2 []
purplish	Emerald, Meyer	3 []
5.5 Time of flowering (in spring) (31)		
early		3 []
medium	Emerald, Meyer	5 []
late		7 []
5.6 Time of senescence of leaves (in autumn) (32)		
early		3 []
medium	Emerald	5 []
late		7 []

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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6. Similar varieties and differences from these varieties

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

Denomination(s) of 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
<i>Example</i>	<i>Stolon color</i>	<i>greenish yellow</i>	<i>red</i>

Comments:

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

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [] No []

(b) Has such authorization been obtained?

Yes [] No []

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

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

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

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

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