E

TG/4/8(proj.1)
ORIGINAL: English
DATE: 2004-06-14

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

RYEGRASS

UPOV code: LOLIU_BOU
LOLIU_MUL
LOLIU_BOU

(*Lolium* spp.)

*

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by an expert from the United Kingdom

to be considered by the
Technical Working Party for Agricultural Crops at its thirty-third session,
to be held in Poznań, Poland, from June 28 to July 2, 2004

Alternative Names:*

Latin	*English*	*French*	*German*	*Spanish*
Lolium perenne L.	Perennial ryegrass	Ray-grass anglais	Deutsches Weidelgras	Ballico perenne, Raygrás inglés
Lolium multiflorum Lam.	Italian ryegrass Westerwolds ryegrass	Ray-grass d'Italie	Welsches Weidelgras, Italienisches Raygras	Ballico italiano, Raygrás italiano
Lolium boucheanum Kunth.	Hybrid ryegrass	Ray-grass hybride	Bastardweidelgras, Oldenburgisches Weidelgras	Ballico híbrido, Raygrás híbrido

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 guidelines("Test Guidelines") should be read in conjunction with document TG/1/3, "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants" (hereinafter referred to as the "General Introduction") and its associated "TGP" documents.

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

TABLE OF CONTENTS PAGE

1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Lolium perenne* L., *Lolium multiflorum* Lam. and *Lolium boucheanum* Kunth . **[+ Festulolium – a request for inclusion from ISF has been agreed]**

2. Material Required

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

2.2 The material is to be supplied in the form of seed.

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

1.5 kg

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should be stated by the applicant.

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

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

3.2 Testing Place

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

3.3 Conditions for Conducting the Examination

3.3.1 The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.3.2 The optimum stage of development for the assessment of each characteristic is indicated by a number in the second column of the Table of Characteristics. The stages of development denoted by each number are described at the end of Chapter 8.

3.3.3 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 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

3.3.4 The recommended type of plot in which to observe the characteristic is indicated by the following key in the second column of the Table of Characteristics:

A: spaced plants
B: row plot
C: special test

3.4 Test Design

3.4.1 Each test should be designed to result in a total of at least 60 spaced plants and 10 meters of row plot which should be divided between 3 replicates.

3.4.2 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 Number of Plants / Parts of Plants to be Examined

3.5.1 Unless otherwise stated, all observations on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observations made on all plants in the test. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

3.6 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.

4.2 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 The assessment of uniformity should be according to the recommendations for cross-pollinated varieties in the General Introduction.

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 tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous 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:

Annual and biennial varieties:

(a) Plant: ploidy (characteristic 1)

Perennial varieties:

(a) Plant: ploidy (characteristic 1)
(b) Plant: time of inflorescence emergence after vernalization (characteristic 9)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

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

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.

6.3 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.

Example varieties are followed by an indication of the botanical types to which they belong. Thus perennial ryegrass (*Lolium perenne* L.) types are followed by (Lp), Italian and Westerwolds ryegrass (*Lolium multiflorum* Lam.) types by (Lm) and hybrid ryegrass (*Lolium boucheanum* Kunth.) by (Lb).

6.5 Legend

(*) Asterisked characteristic – see Chapter 6 (Section 6.1.2)

QL Qualitative characteristic – see Chapter 6 (Section 6.3)
QN Quantitative characteristic – see Chapter 6 (Section 6.3)
PQ Pseudo-qualitative characteristic – see Chapter 6 (Section 6.3)

MG: single measurement of a group of plants or parts of plants – see Section 3.3.3
MS: measurement of a number of individual plants or parts of plants – see Section 3.3.3
VG: visual assessment by a single observation of a group of plants or parts of plants – see Section 3.3.3
VS: visual assessment by observation of individual plants or parts of plants – see Section 3.3.3

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

(+) See Explanations on the Table of Characteristics in Chapter 8.

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

Char No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. (*)	**MS C**	**Plant: ploidy**					
QL		diploid					2
		tetraploid					4
2.	**VS A, B**	**Plant: vegetative growth habit (without vernalization)**					
QN	**(a)**	erect					1
		semi erect					3
		medium					5
		semi prostrate					7
		prostrate					9
3. (+)	**VS A, B**	**Plant: tendency to form inflorescences (without vernalization)**					
QN		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9

Char No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4. (*)	**MS A, B**	**Plant: time of inflorescence emergence (without vernalization – annual types only)**					
QN	**(b)**	very early					1
		early					3
		medium					5
		late					7
		very late					9
5. (+)	**VS B**	**Leaf: color**					
QN		very light green					1
		light green					3
		medium green					5
		dark green					7
		very dark green					9
6.	**VS A, B**	**Plant: vegetative growth habit (after vernalization)**					
QN	**(a)**	erect					1
		semi erect					3
		medium					5
		semi prostrate					7
		prostrate					9

Char No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
7. (+)	**MS A, B**	**Plant : natural height (after vernalization)**					
QN		very short					1
		short					3
		medium					5
		tall					7
		very tall					9
8.	**MS A**	**Plant: width (after vernalization)**					
QN		very narrow					1
		narrow					3
		medium		**NEW PROPOSAL**			5
		wide					7
		very wide					9
9. (*)	**MS A, B**	**Plant: time of inflorescence emergence (after vernalization – biennial and perennial types only)**					
QN	**(b)**	very early					1
		early					3
		medium					5
		late					7
		very late					9

Char No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
10.	**MS A**	**Plant: natural height at inflorescence emergence**					
QN	**(c)**	very low					1
		low					3
		medium					5
		tall					7
		very tall					9
11.	**MS A**	**Plant: width**					
QN	**(c)**	very narrow					1
		narrow					3
		medium		NEW PROPOSAL			5
		wide					7
		very wide					9
12. (*)	**MS A**	**Flag leaf: length**					
QN	***(c)***	very short					1
		short					3
		medium					5
		long					7
		very long					9

Char No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
13. (*)	**MS A**	**Flag leaf: width**					
QN	**(c)**	very narrow					1
		narrow					3
		medium					5
		broad					7
		very broad					9
14. (*)	**MS A**	**Plant: length of longest stem, inflorescence included (when fully expanded)**					
QN	**(d)**	very short					1
		short					3
		medium					5
		long					7
		very long					9
15.	**MS A**	**Inflorescence: length**					
QN	**(d)**	very short					1
		short					3
		medium					5
		long					7
		very long					9

Char No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
16.	**MS A**	**Inflorescence: number of spikelets**					
QN	**(d)**	very few					1
		few					3
		medium					5
		many					7
		very many					9
17.	**MS A**	**Inflorescence: length of glume**					
QN	**(d)**	very short					1
		short					3
		medium		NEW PROPOSAL			5
		long					7
		very long					9
18.	**MS A**	**Inflorescence: length of basal spikelet**					
QN	**(d)**	very short					1
		short					3
		medium		NEW PROPOSAL			5
		long					7
		very long					9

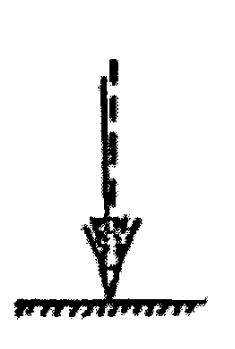
8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

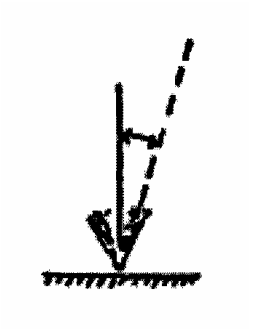
(a) Ad. 2: Plant: vegetative growth habit (without vernalization)
Ad. 6: Plant: vegetative growth habit (after vernalization)

Characteristic 2 may be recorded on annual, biennial and perennial types, during the same growing season as when the trials are planted. Characteristic 6 should only be recorded on biennial and perennial types, during the second growing season, 4 weeks after growth has started in the earliest variety.

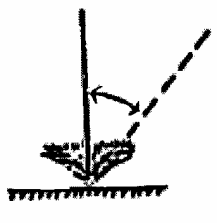
The observations should be made visually from the attitude of the leaves of the plant as a whole. The angle formed by the imaginary line through the region of greatest leaf density and the vertical should be used.



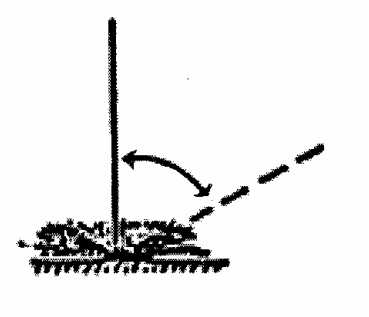
1
erect



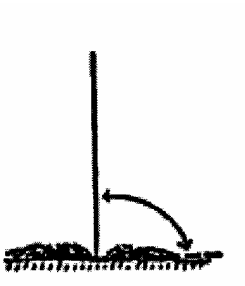
3
semi erect



5
medium



7
semi prostrate



9
prostrate

(b) Ad. 4: Plant: Time of inflorescence emergence (without vernalization)

This characteristic should normally only be recorded on annual types.

Ad. 9: Plant: Time of inflorescence emergence (after vernalization)

This characteristic should normally only be recorded on biennial and perennial types.

Plots with spaced plants and row plots should be observed at least twice per week and more frequently if there is any need to do so.

Plots with spaced plants

The date of inflorescence emergence of each single plant should be observed. A single plant is considered to have headed when the tip of three inflorescences can be seen protruding from the flag leaf sheath. From the single plant data, a mean date per plot and a mean date per variety is obtained.

Row plots

At each observation date the average plot stage should be expressed in one of the following growth stages:

1) Boot swollen
2) Tip of inflorescence just visible
3) ¼ of inflorescence emerged
4) ½ of inflorescence emerged

The date of inflorescence emergence is the date at which the average plot stage 2 has been reached. This date should, if necessary, be obtained by interpolation.

(c) Ad. 10: Plant: natural height at inflorescence emergence
Ad. 11: Plant: width
Ad. 12: Flag leaf: length
Ad. 13: Flag leaf: width

These characteristics should be recorded at the time of inflorescence emergence, that is, at the same time as Characteristic 4 for annual types and Characteristic 9 for biennial and perennial types.

(d) Ad 14: Plant: length of longest stem, inflorescence included (when fully expanded)
Ad. 15: Inflorescence: length
Ad. 16: Inflorescence: number of spikelets
Ad. 17: Inflorescence: length of glume
Ad. 18: Inflorescence: length of basal spikelet

These characteristics should be recorded at the same time, when the inflorescence is fully expanded and using the same stem for all.

8.2 Explanations for individual characteristics

Ad. 3: Plant: tendency to form inflorescences (without vernalization)

The number of plants showing at least three inflorescences should be recorded for each variety. To be assessed on one occasion on the whole trial when the varieties are judged to have reached their full expression of this characteristic.

Ad. 5: Leaf: color

Annual types:

Leaf color should be recorded at beginning of inflorescence emergence.

Biennial and perennial types:

Leaf color should be recorded during the same growing season as when the trials are planted.

Ad. 7: Plant: natural height (after vernalization)

Natural height should be observed during the second growing season, 4 weeks after growth has started in the earliest variety.

9. Literature

Baltjes, H.J., Klein Geltink, D.J.A., Nienhuis, K.H. and Luesink, B. 1985: Linking Distinctiveness and Description of Varieties, Journal of the National Institute of Agricultural Botany, GB, 17, pages 9-19

Camlin, M.S., Watson, S., Waters, B.G. and Weatherup, S.T.C. 2001: The potential for management of reference collections in herbage variety registration trials using a cyclic planting system for reference varieties, Plant Varieties and Seeds, GB, 14, pages 1-14

Hawkins, R.P. 1958: The Classification of the Strains (Varieties) of Herbage Plants, Journal of the National Institute of Agricultural Botany, GB, 9, pages 434-449

Patterson, H.D. and Weatherup S.T.C. 1984: Statistical Criteria for Distinctness between Varieties of Herbage Crops, Journal of Agricultural Science, Cambridge, GB, 102, pages 59-68

Tyler, B.F., Hayes, J.D. and Ellis Davies, W. 1985: IBPGR/CEC Descriptive List for Forage Grasses, International Board for Plant Genetic Resources (IBPGR), 83/90

Weatherup, S.T.C. 1980: Statistical Procedures for Distinctness, Uniformity and Stability Trials, Journal of Agricultural Science, Cambridge, GB, 94, page 31-46

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 (please indicate the relevant species):

1.1.1 *Latin Name*	*Lolium perenne* L.	[ ]
1.1.2 Common Name	Perennial ryegrass	
1.2.1 *Latin Name*	*Lolium Multiflorum* Lam.	[ ]
1.2.2 Common Name	Italian ryegrass	
1.3.1 *Latin Name*	*Lolium Multiflorum* Lam.	[ ]
1.3.2 Common Name	Westerwolds (annual) ryegrass	
1.4.1 *Latin Name*	*Lolium boucheanum* Kunth.	[ ]
1.4.2 Common Name	Hybrid ryegrass	

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	

Breeder (if different from applicant)

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

3. Proposed denomination and breeder's reference

Proposed denomination (if available) [ ]

Breeder's reference [ ]

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

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)
(b) partially known cross [ ]
(please state known parent variety(ies))
(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery and development [ ]
(please state where and when discovered and how developed)

4.1.4 Other [ ]
(please provide details)

4.2 Method of propagating the variety

[#] Authorities may allow certain parts of this information to be given 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
5.1 (1)	**Plant: ploidy**		
	diploid		2
	tetraploid		4
5.2 (4)	**Time of inflorescence emergence (without vernalization - annual types only)**		
	very early		1
	early		3
	medium		5
	late		7
	very late		9
5.3 (9)	**Time of inflorescence emergence (after vernalization - biennial and perennial types only)**		
	very early		1
	early		3
	medium		5
	late		7
	very late		9
5.4 (14)	**Plant: length of longest stem, inflorescence included (when fully expanded)**		
	very short		1
	short		3
	medium		5
	long		7
	very long		9

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

6. Similar varieties and differences from these varieties

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

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example			

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

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 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 of 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]