



PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Botanical name	English name
<i>Lolium perenne</i> L.	Perennial ryegrass
<i>Lolium multiflorum</i> Lam. spp. <i>italicum</i> (A. Br.) Vokart; <i>Lolium multiflorum</i> Lam. spp. <i>non alternativum</i>	Italian ryegrass
<i>Lolium multiflorum</i> Lam. var. <i>westerwoldicum</i> Wittmt; <i>Lolium multiflorum</i> Lam. spp. <i>alternativum</i>	Westerwolds ryegrass
<i>Lolium boucheanum</i> Kunth ; <i>Lolium x hybridum</i> Hausskn.	Hybrid ryegrass
<i>Lolium rigidum</i> Gaudin.	Stiff darnel, Wimmera ryegrass

RYEGRASS

UPOV Species Codes:
LOLIU_PER; LOLIU_MUL_ITA; LOLIU_MUL_WES; LOLIU_BOU; LOLIU_RIG

Adopted on 23/06/2011

Entry into force on 23/06/2011

I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation (EC) No. 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/4/8 dated 05/04/2006 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of ***Lolium perenne* L., *Lolium multiflorum* Lam.ssp. *italicum* (A. Br.) Volkart, *Lolium multiflorum* Lam. var. *westerwoldicum*, *Lolium boucheanum* Kunth. and *Lolium rigidum* Gaudin.**

II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the Examination Office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection of the Examination Office as the definitive sample of the candidate variety.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO if no plant material has been received. However, if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Seed requirements

Information with respect to closing dates and submission requirements of plant material for the technical examination of varieties can be found on the CPVO web site (www.cpvo.europa.eu).

Quality of seed material: The minimum requirements should not be less than the standards laid down in Council Directive 66/401/EEC.

Seed treatment: The plant material must not have undergone any treatment unless the CPVO and the Examination Office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Labelling of sample:

- Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination reference (if known)
- Name of applicant
- The phrase "On request of the CPVO".

III CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation (EC) No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.

The composition of the variety collection in each Examination Office depends on the ecological conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other offices involved in DUS-testing of ryegrass. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation (EC) No. 1239/95, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics that could be used for grouping are the following (CPVO numbering; G for grouping in the table of characteristics).

Lolium multiflorum Lam. var. *westerwoldicum* (Lmw) and *Lolium rigidum* Gaudin (Lr):

- (a) Plant: ploidy (characteristic 1)
- (b) Only varieties of Lmw and Lr: Plant: time of inflorescence emergence (without vernalization) (characteristic 8)
- (c) Plant: length of longest stem, inflorescence included (when fully expanded) (characteristic 16)

Lolium perenne L.(Lp), *Lolium multiflorum* Lam. ssp. *italicum* (A. Br.) Volkart (Lmi) and *Lolium boucheanum* Kunth (Lb)

- (a) Plant: ploidy (characteristic 1)
- (b) Only varieties of Lp, Lmi and Lb: Plant: time of inflorescence emergence (after vernalization) (characteristic 10)
- (c) Plant: length of longest stem, inflorescence included (when fully expanded) (characteristic 16)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth.

The test design is as follows:

As a minimum, each test should include at least:

60 spaced plants which should be divided between at least 3 replicates.

In addition the test may include 8 meters of row plot which should be divided between at least 2 replicates. The density of the seed should be such that around 200 plants/meter can be expected and the satisfactory establishment should be checked shortly after emergence.

Unless otherwise indicated, 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.

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

6. Special tests

In accordance with Article 83(3) of Council Regulation (EC) No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation (EC) No. 2100/94.

Qualitative characteristics:

In the case of characteristics which show discrete discontinuous states of expression, a difference between two varieties is clear if the respective characteristics have expressions which fall into two different states.

Quantitative characteristics:

Characteristics which show a continuous range of expression from one extreme to the other may be either visually observed or measured.

In the case of characteristics assessed by a single observation of a group of plants or parts of plants (VG, MG), a difference between two varieties is clear if the expression of the respective characteristics differs by at least the span of one note, taking into account the variability observed within the varieties.

In the case of characteristics assessed by observations of individual plants or parts of plants (VS, MS) or by repeated measurements of groups of plants (MG), distinctness should be assessed by the combined over years distinctness analysis (COYD). The probability level for the COYD criterion should be 1% or less ($p < 0.01$) in a test over either two or three growing cycles.

If the conditions for the application of the COYD analyses are not fulfilled, distinctness should be assessed using the 2x1% method.

b) **Uniformity**

The variability within the variety should not exceed the variability of comparable varieties already known.

In case of observation of a group of plants or parts of plants (VG, MG) for the assessment of distinctness, uniformity should be assessed on the basis of visual observation of off-types.

In case of observation of individual plants or parts of plants (VS, MS) for the assessment of distinctness, uniformity should be assessed by using COYU or other appropriate statistical methods.

If uniformity is assessed by the combined over years uniformity method (COYU)

- the candidate variety which has completed two years of test, can be accepted as uniform when its combined over years uniformity (COYU) is not significantly greater than that of the appropriate reference varieties at the 1% ($P=0.01$) significance level.

- the candidate variety which has completed three years of test, can be accepted as uniform when its combined over years uniformity (COYU) is not significantly greater than that of the appropriate reference varieties at the 0.1% ($P=0.001$) significance level.

If the conditions for the application of the COYU analyses are not fulfilled, uniformity should be assessed using the relative variance method. For a sample size of 60 plants, the threshold level should be 1.6 x variance of the comparable varieties.

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity. Seed samples of further submissions included in any test must show the same expression of characteristics as the material originally supplied.

IV REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing cycles but in some cases three growing cycles may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the Examination Office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V LIAISON WITH THE APPLICANT

If problems arise during the course of the test the CPVO should be informed so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

VI ENTRY INTO FORCE

The present protocol enters into force on **23/06/2011**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the revised Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

Introduction to the Table of Characteristics

1. Legend

1.1 Type of characteristic

QL: Qualitative characteristic
QN: Quantitative characteristic

1.2 Method of observation

MG: Measurement of a group of plants or plant parts
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 plant parts
VS: Visual assessment by observation of individual plants or plant parts

If more than one method of observation is indicated for a specific characteristic, the examination office has to choose the most appropriate method under its conditions. The characteristic should not be assessed twice.

1.3 The recommended type of plot in which to observe the characteristic

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

If more than one type of plot is indicated for a specific characteristic, the examination office has to choose the most appropriate plot type under its conditions. The characteristic should not be assessed twice.

1.4 Species abbreviations for example varieties:

(Lp): *Lolium perenne* L.
(Lmi): *Lolium multiflorum* Lam. *italicum* (A. Br.) Volkart
(Lmw): *Lolium multiflorum* Lam. var. *westerwoldicum* Wittm
(Lb): *Lolium boucheanum* Kunth.
(Lr): *Lolium rigidum* Gaudin.

1.5 Further abbreviations in the table of characteristics

(a)-(e) Explanations covering several characteristics
(+) Explanations for individual characteristics

VI - TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTION

CPVO N°	UPOV N°	Stage, ¹ Method	Characteristics	Examples ²	Note
1. (+) QL G	1.	C	Plant: ploidy		
			diploid	Bargold (Lp), Barsilo (Lb)	2
			tetraploid	Tivoli (Lp), Fabio (Lmi)	4
2. QN	2.	20-29 VS A (a)	Plant: vegetative growth habit (without vernalization)		
			erect	Solita (Lmi)	1
			semi-erect	Lemtal (Lmi)	3
			medium	Jeanne (Lmi), Jumbo (Lp)	5
			semi-prostrate	Titus (Lp), Belida (Lp)	7
			prostrate	Citius (Lp)	9
3. QN	5.	20-29 VG A / VG B	Leaf: intensity of green colour (without vernalization)		
			very light		1
			light	Abermont (Lp), Superstar (Lp)	3
			medium	Tivoli (Lp), Barsilo (Lb)	5
			dark	Adeline (Lp), Greenway (Lp)	7
			very dark	Polarstar (Lp)	9
4. QN	6.	30 MS A / VS A (+)	Plant: width (after vernalization)		
			very narrow	Aberelf (Lp)	1
			narrow	Disco (Lp), Hamlet (Lp)	3
			medium	Jeanne (Lmi)	5
			wide	Lacerta (Lp), Fanal (Lp)	7
			very wide	Pimpernel (Lp)	9

¹ See explanations in Annex 1.

² Example varieties are given as an indication, others may be used.

CPVO N°	UPOV N°	Stage, ¹ Method	Characteristics	Examples ²	Note
5.	7.	30-39 VS A / VG B	Plant: vegetative growth habit (after vernalization)		
QN		(a)	erect	Solita (Lmi)	1
			semi-erect	Grasslands Nui (Lp), Lemtal (Lmi) Lemnos (Lmw), Barsilo (Lb)	3
			medium	Palmer (Lp), Texy (Lb), Lacerta (Lp), Enduro (Lb)	5
			semi-prostrate	Tivoli (Lp),	7
			prostrate	Fabula (Lp)	9
6.	8.	30-39 MS A / VG B	Plant: height (after vernalization)		
QN			very short	Aberelf (Lp)	1
			short	Titus (Lp)	3
			medium	Tivoli (Lp), Fabio (Lmi), Adeline (Lp)	5
			tall	Fox (Lmi), Lacerta (Lp)	7
			very tall		9
7.		30-39 VG A / VG B	Only varieties of Lp, Lmi and Lb: Leaf: intensity of green colour (after vernalization)		
QN				AberDart (Lp)	3
				Vesuve (Lp), Bellem (Lmi), Premiun (Lp), Gemini (Lmi)	5
				Mondora (Lmi), Verdi (Lp) Boxer (Lb)	7
				Polarstar (Lp)	9

CPVO N°	UPOV N°	Stage, ¹ Method	Characteristics	Examples ²	Note
8.	9.	50 MS A	<u>Only varieties of Lmw and Lr: Plant : time of inflorescence emergence (without vernalization)</u>		
QN		(b)	very early	Grazer (Lmw)	1
			early	Lifloria (Lmw), Libonus (Lmw)	3
			medium	Elunaria (Lmw)	5
			late	Advance (Lmw), Vivaro (Lmw)	7
G			very late	Koga (Lmw), Telga (Lmw)	9
9.	10.	50 VS A VG B	Plant: tendency to form inflorescences (without vernalization)		
(+)			absent or very weak	Bargold (Lp), Barmultra (Lmi), Enduro (Lb)	1
QN			weak	Arvicola (Lp), Fox (Lmi), Gemini (Lmi)	3
			medium	Faveur (Lp), Ligrande (Lmi) Barsilo (Lb)	5
			strong	Lemtal (Lmi)	7
			very strong	Weldra (Lmw), Arolus (Lp)	9
10.	11.	50 MS A	<u>Only varieties of Lp, Lmi and Lb: Plant: time of inflorescence emergence (after vernalization)</u>		
QN		(b)	very early	Ivana (Lp)	1
			early	Lacerta (Lp), Enduro (Lb)	3
			medium	Greenway (Lp), Boxer (Lb)	5
			late	Chagall (Lp)	7
G			very late	Cancan (Lp)	9
11.	12.	50 - 56 MS A	Plant: natural height at inflorescence emergence		
QN		(c)	very short	Loretta (Lp)	1
			short	Titus (Lp)	3
			medium	Cancan (Lp), Ligrande (Lmi)	5
			tall	Lemtal (Lmi), Lacerta (Lp)	7
			very tall	Lipo (Lmi)	9

CPVO N°	UPOV N°	Stage, ¹ Method	Characteristics	Examples ²	Note
12.		50 VS A / MS A	Plant: growth habit at inflorescence emergence		
QN				Fabio (Lmi)	3
				Isabel RvP (Lp), Premium (Lp)	5
13.	14.	50 MS A	Flag leaf: length		
QN		(c)	very short	Brightstar (Lp)	1
			short	Sauvignon (Lp), Bargold (Lp)	3
			medium	Lipresso (Lp), Fastyl (Lmi)	5
			long	Ibex (Lb), Twins (Lp), Acento (Lp)	7
			very long		9
14.	15.	50 MS A	Flag leaf: width		
QN		(c)	very narrow	Bargold (Lp)	1
			narrow	Loretta (Lp)	3
			medium	Lipresso (Lp), Fennema (Lp) Lemnos (Lmw)	5
			broad	Eurostar (Lp), Lacerta (Lp) Barsilo (Lb)	7
			very broad	Lipo (Lmi)	9
15.	16.	50 MS A	Flag leaf: length/width ratio		
QN		(c)	very low	Ivana (Lp)	1
			low	AberElf (Lp), Lacerta (Lp)	3
			medium	Fabio (Lmi), Mondial (Lp)	5
			high	Twystar (Lp)	7
			very high	Cancan (Lp)	9

CPVO N°	UPOV N°	Stage, ¹ Method	Characteristics	Examples ²	Note
16.	17.	60-68 MS A	Plant: length of longest stem, inflorescence included (when fully expanded)		
(+)		(d)	very short	Brightstar (Lp)	1
QN			short	Loretta (Lp), Grazer (Lmw)	3
			medium	Cancan (Lp)	5
			long	Fox (Lmi), Limbos (Lp)	7
G			very long	Lipo (Lmi), Fleurial (Lb)	9
17. (+)	18.	60-68 MS A	Plant: length of upper internode		
QN		(d)	very short	Abersprite (Lp)	1
			short	Adeline (Lp)	3
			medium	Cancan (Lp), Lental (Lmi)	5
			long	Montblanc (Lmi), Acrobat (Lb)	7
			very long	Pirol (Lb)	9
18.	19.	60-68 MS A	Inflorescence: length		
QN		(d)	very short	Sunbright (Lp)	1
			short	Bellem (Lmi), Bargold (Lp)	3
			medium	Barmega (Lmi), Libonus (Lmw), Vigor (Lp)	5
			long	Lacerta (Lp), Acrobat (Lb)	7
			very long	Fabio (Lmi)	9
19.	20.	60-68 MS A	Inflorescence: number of spikelets		
QN		(d)	very few		1
			few	Abersprite (Lp), Bargold (Lp)	3
			medium	Acento (Lp), Fabio (Lmi) Lental (Lmi), Barsilo (Lb)	5
			many	Fortimo (Lb)	7
			very many		9

CPVO N°	UPOV N°	Stage, ¹ Method	Characteristics	Examples ²	Note
20. (+) QN	21.	60-68 MS A (d)	Inflorescence: density		
			very lax	Jaran (Lp)	1
			lax	Leon (Lp), Ligrande (Lmi)	3
			medium	Meritra (Lmi), Libonus (Lmw)	5
			dense	Lacerta (Lp)	7
			very dense		9
21. QN	22.	60-68 MS A (d)	Inflorescence: length of outer glume on basal spikelet		
			very short	Lema (Lmi)	1
			short	Prestyl (Lmi), Bareuro (Lp)	3
			medium	Fennema (Lp), Ibex (Lb)	5
			long	Meradonna (Lp), Lemnos (Lmw) Texy (Lb)	7
			very long	Litempo (Lp)	9
22. QN	23.	60-68 MS A (d)	Inflorescence: length of basal spikelet excluding awn		
			very short	AberElf (Lp)	1
			short	Sunbright (Lp), Montreux (Lp)	3
			medium	Barprisma (Lmi), Lipresso (Lp)	5
			long	Edda (Lp), Libonus (Lmw), Storm (Lb)	7
			very long	Litempo (Lp)	9

ANNEXES TO FOLLOW

	<u>PAGE</u>
ANNEX I	
Explanations and methods.....	15
Decimal code for growth stages.....	18
ANNEX II	
Technical Questionnaire	19

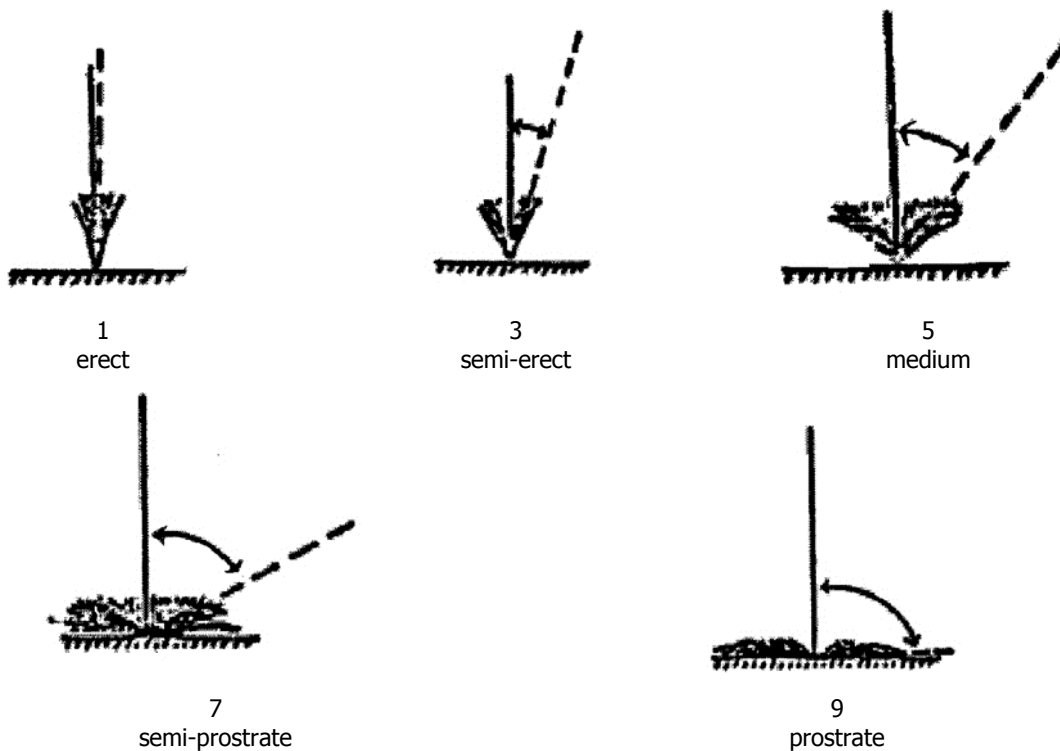
ANNEX I

1. Explanations on the Table of Characteristics

1.1 *Explanations covering several characteristics*

(a) Growth habit

Characteristic 2 may be recorded during the growing season in which the trials are planted. 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.



(b) Time of inflorescence emergence

Timing of observations will depend upon the time of planting. Spaced plants or row plots should be observed at least twice per week.

A: Plots with spaced plants

The time 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 (Growth Stage DC 50). From the single plant data, a mean date per plot and a mean date per variety is obtained.

B: Row plots

The time of inflorescence emergence is the date at which the average plot stage DC 54 has been reached. This date should – if necessary– be obtained by interpolation. At each observation date, the average plot stage should be expressed in one of the following growth stages:

DC 50	First spikelet of inflorescence just visible
DC 52	25% of the inflorescence emerged (across all stems)
DC 54	50% of the inflorescence emerged (across all stems)
DC 56	75% of the inflorescence emerged (across all stems)

- (c) To be recorded on each individual plant at the time of inflorescence emergence (Growth Stage DC 50), that is at the same time as Characteristic 9 for *Lolium multiflorum* Lam. var. *westerwoldicum* and *Lolium rigidum* Gaudin. and Characteristic 11 for *Lolium perenne* L., *Lolium multiflorum* Lam. ssp. *italicum* (A. Br.) Volkart and *Lolium boucheanum* Kunth.
- (d) Observations should be made on the longest stem.

2.2 Explanations for individual characteristics

Ad. 1: Plant: ploidy

The ploidy of the plant can be determined either by standard cytological methods or by observing the occurrence of 5-band genotypes (which are present only in tetraploid varieties) in phosphoglucosomerase (PGI) isoenzyme electrophoresis.

Ad. 4: Plant: width

To allow for irregular plant shapes (for example due to wind shaping effects) the plant width is determined by taking two measurements (MS A) or by making two visual observations (VS A) of the diameter across the plant at right angles to each other and then using the average of these two figures as the plant width.

Ad. 9: 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. 16: Plant: length of longest stem, inflorescence included (when fully expanded)

To be recorded in the field from ground level, when the inflorescence is fully expanded.

Ad. 17: Plant: length of upper internode

To be measured from the top node to the base of the inflorescence.



Ad. 21: Inflorescence: density

This characteristic is calculated by dividing characteristic 19 (Inflorescence: length) by characteristic 20 (Inflorescence: number of spikelets).

2. Growth stages for grasses

All characteristics should be recorded at the appropriate time for the plant concerned. Growth stages of grasses are indicated by decimal codes which are derived from the decimal code for the growth stages of cereals (Zadoks, et al., 1974). This decimal code is in close conformity with the BBCH-code (Meier, 1997).

Seedling growth (seedling: one shoot)

- DC 10 First leaf through coleoptile
- DC 15 Five leaves unfolded
- DC 19 Nine or more leaves unfolded

Tillering

- DC 20 Main shoot only (beginning of tillering)
- DC 23 Main shoot and 3 tillers
- DC 25 Main shoot and 5 tillers
- DC 29 Main shoot and 9 or more tillers

Stem elongation

- DC 30 Pseudo-stem erection (formed by sheaths of leaves)
- DC 31 First node detectable (early stem extension across all stems)
- DC 35 Fifth node detectable (50 % extension across all stems)
- DC 39 Flag leaf ligula/collar just visible (pre-boot stage)

Booting

- DC 41 Flag leaf sheath extending (little enlargement of the inflorescence, early boot-stage)
- DC 45 Boots swollen (late-boot stage)
- DC 47 First leaf sheath opening
- DC 49 first awns visible (in awned forms only)

Inflorescence emergence (mostly non-synchronous)

- DC 50 First spikelet of inflorescence just visible
- DC 52 25 % of the inflorescence emerged (across all stems)
- DC 54 50 % of the inflorescence emerged (across all stems)
- DC 56 75 % of the inflorescence emerged (across all stems)
- DC 58 Emergence of inflorescence completed

Anthesis (mostly non-synchronous)

- DC 60 Beginning of anthesis
- DC 64 Anthesis half-way
- DC 68 Anthesis complete

ANNEX II



TECHNICAL QUESTIONNAIRE

to be completed in connection with an application for Community Plant Variety Rights
Please answer all questions. A question without any answer will lead to a non-attribution
of an application date. In cases where a field / question is not applicable, please state so.

1. **Botanical taxon:** Name of the genus, species or sub-species to which the variety belongs and common name

Lolium

RYEGRASS

Please tick the relevant box of the species to which your application belongs:

- Lolium perenne* L.
- Lolium multiflorum* Lam. ssp. *italicum* (A. Br.) Volkart
- Lolium multiflorum* Lam. var. *westerwoldicum* Wittm
- Lolium boucheanum* Kunth.
- Lolium rigidum* Gaudin.

2. **Applicant(s):** Name(s) and address(es), phone and fax number(s), Email address, and where appropriate name and address of the procedural representative

3. **Variety denomination**

a) Where appropriate proposal for a variety denomination:

b) Provisional designation (breeder's reference):

4. Information on origin, maintenance and reproduction of the variety

4.1 Origin

- (a) Seedling (indicate parent varieties)[]
- (b) Mutation (indicate parent variety)[]
- (c) Discovery (indicate where, when and how the variety has been developed)[]
- (d) Other (please specify)[]

4.2 Method of propagation

- (a) Cuttings[]
- (b) *In vitro* propagation[]
- (c) Seed[]
- (d) Other (please specify):[]

4.3 Other information

In the case of seed propagated varieties: method of production:

- (a) Self-pollinated.....[]
- (b) Cross-pollinated (please give details)[]
- (c) Hybrid (please give details)[]

4.4 Geographical origin of the variety: the region and the country in which the variety was bred or discovered and developed			
5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in the CPVO Protocol; please mark the state of expression which best corresponds).			
Characteristics		Example varieties	Note
5.1 (1)	Plant: ploidy		
	diploid	Bargold (Lp), Barsilo (Lb)	2 []
	tetraploid	Fabio (Lmi), Tivoli (Lp)	4 []
5.2 (8)	<u>Only varieties of Lmw and Lr:</u> Plant: time of inflorescence emergence (without vernalization)		
	very early	Grazer (Lmw)	1 []
	very early to early		2 []
	early	Lifloria (Lmw), Libonus (Lmw)	3 []
	early to medium		4 []
	medium	Elunaria (Lmw)	5 []
	medium to late		6 []
	late	Advance (Lmw), Vivaro (Lmw)	7 []
	late to very late		8 []
	very late	Koga (Lmw), Telga (Lmw)	9 []
5.3 (10)	<u>Only varieties of Lp, Lmi and Lb:</u> Plant: time of inflorescence emergence (after vernalization)		
	very early	Ivana (Lp)	1 []
	very early to early		2 []
	early	Lacerta (Lp), Enduro (Lb)	3 []
	early to medium		4 []
	medium	Greenway (Lp), Boxer (Lb)	5 []
	medium to late		6 []
	late	Chagall (Lp)	7 []
	late to very late		8 []
	very late	Cancan (Lp)	9 []

Characteristics		Example varieties	Note
5.4 (16)	Plant: length of longest stem, inflorescence included (when fully expanded)		
	very short	Brightstar (Lp)	1 []
	very short to short		2 []
	short	Loretta (Lp), Grazer (Lmw)	3 []
	short to medium		4 []
	medium	Cancan (Lp)	5 []
	medium to long		6 []
	long	Fox (Lmi), Limbos (Lp)	7 []
	long to very long		8 []
very long	Lipo (Lmi), Fleurial (Lb)	9 []	
6. Similar varieties and differences from these varieties:			
Denomination of similar variety	Characteristic in which the similar variety is different ¹⁾	State of expression of similar variety	State of expression of candidate variety
<p>_____</p> <p>¹⁾ In the case of identical states of expressions of both varieties, please indicate the degree of the difference</p>			
7. Additional information which may help to distinguish the variety			
7.1 Resistance to pests and diseases			
[] YES, please specify			
[] NO			
7.2 Intended utilisation			
[] Amenity			
[] Forage			

7.3 Special conditions for the examination of the variety

YES, please specify

NO

7.4 Other information

YES, please specify

NO

8. GMO-information required

The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive EC/2001/18/EC of 12/03/2001.

YES NO

If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation 2100/94 does not pose risks to the environment according to the norms of the above-mentioned Directive.

9. Information on plant material to be examined

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 or pesticide) Yes No
- (c) Tissue culture Yes No
- (d) Other factors Yes No

Please provide details of where you have indicated "Yes":

9.3 In case the variety is infected with endophytes please indicate for information purpose the level of infection

Please tick the relevant category

✓	Category	% endophytes
<input type="checkbox"/>	I	< 10%
<input type="checkbox"/>	II	10 < % < 85
<input type="checkbox"/>	III	>85%

10. Possible place of the technical examination

In case the CPVO needs to arrange a technical examination for this candidate variety, there might be more than one examination office entrusted by the CPVO suitable to grow your variety. In this case, the Office will decide on the place of the technical examination but you might wish to express here a preference in respect of an examination office. The available entrusted examination offices for that species can be found in the S2 Gazette under <http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette>

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date

Signature

Name

[End of document]