

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Poa pratensis L.

KENTUCKY BLUEGRASS

UPOV Code: POAAA_PRA

Adopted on 15/03/2017

Entry into force on 15/04/2017

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CPVO-TP/033/1

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1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of *Poa pratensis* L.

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 http://www.upov.int/en/publications/intro_dus.htm), its associated TGP documents (<http://www.upov.int/en/publications/tgp/>) and the relevant UPOV Test Guideline TG/33/7 dated 04/09/2014 <http://www.upov.int/edocs/tgdocs/en/tg033.pdf> for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **15.04.2017**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the 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.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report. If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

If problems arise during the course of the test the CPVO should be informed immediately 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.

1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette> in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- 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.

2.3 Informing about problems on the submission of material

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

The minimum duration of tests should normally be two independent growing cycles. The two independent growing cycles should be in the form of separate plantings.

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"

http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_9_1.pdf.

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.

The optimum stage of development for the assessment of each characteristic is indicated by a number in the third column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.2.

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: greenhouse 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.

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 30 spaced plants, for apomictic varieties, and at least 60 plants, for non-apomictic varieties, which should be divided between at least 2 replicates. In addition, the test may include 8 meters of row plot which should be divided between at least 2 replicates. The sowing density should be such that around 200 plants per meter can be expected.

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 Additional tests

In accordance with Article 83(3) of Council Regulation 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, an additional test may be undertaken providing that a technically acceptable test procedure can be devised.

Additional 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.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection

The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material

The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

3.6.3 Range of the variety collection

The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO.

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection

The inventory shall take into account the list of protected varieties and the official, or other, registers of varieties, in particular:

The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue.

3.6.5 Maintenance and renewal/update of a living variety collection

The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

4.1 Distinctness

4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_9_1.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

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 Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

Decision standards

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p < 0.01$) in a test over either two or three years.

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

If distinctness is assessed using the 2 x 1% criterion, the varieties need to be significantly different in the same direction at the 1% level in at least two out of three years in one or more measured characteristics. The tests in each year are based on Student's two-tailed t-test of the differences between variety means with standard errors estimated using the residual mean square from the analysis of the variety x replicate plot means.

4.1.4 Number of plants/parts of plants to be examined

4.1.4.1 Apomictic varieties: Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 20 plants or parts taken from each of 20 plants and any other observations made on all plants in the test, disregarding any off-type plants.

4.1.4.2 Non-apomictic 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 observations made on all plants in the test.

4.1.4.3 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.

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 third column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "Observation of characteristics"):

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

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

"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. colour 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

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_10_1.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

4.2.1 Apomictic varieties

For the assessment of uniformity a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 30 plants, 2 off-types are allowed.

4.2.2 Non-apomictic varieties

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 1% significance level or less ($p < 0.01$) in a test over two years.

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 0.1% significance level or less ($p < 0.001$) in a test over three years.

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

4.3 Stability

4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_11_1.pdf)

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.

Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed 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) Plant: time of inflorescence emergence (characteristic 8)
- b) Plant: natural height when fully expanded (characteristic 11)

5.4 If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 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 or by specific legislation on plant health. In the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

States of expression and corresponding notes

In the case of qualitative and pseudo-qualitative characteristics, 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 Example Varieties

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

6.3 Legend

For the CPVO N° column:

G	Grouping characteristic	– see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	See Explanations on the Table of Characteristics in Chapter 8.1	

For the UPOV N° column:

The numbering of the characteristics is provided as a reference to the ad hoc UPOV guideline.

(*)	UPOV Asterisk characteristic – Characteristics that are important for the international harmonization of variety descriptions.
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For the column "stage, method":

23 – 68	See Explanations on the Table of Characteristics in Chapter 8.2
MG, MS, VG, VS	Method of observation – see Chapter 4.1.5
A, B, C	Type of plot – see Chapter 3.3

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note		
1.	1.	23-25 C	Leaf sheath: anthocyanin coloration				
			QN	VS	absent or very weak	Baron	1
					weak	Oxford	3
					medium	Evora	5
			strong	Limagie	7		
2.	2.	25-29	Plant: natural height <u>before stem elongation</u>				
			QN	MS A/ MG B	short	Limousine	3
					medium	Limerick	5
			tall	Bariris	7		
3.	3.	25-29	Leaf: width				
			QN	MS A/ VS A/ VG B	narrow	Limousine	3
					medium	Conni	5
			broad	Miracle	7		
4.	4.	29	Plant: growth habit <u>without vernalization</u>				
			(+)	VS A/ VG B	erect		1
			QN		semi-erect	Julia	3
					intermediate	Limerick	5
					semi-prostrate	Yvette	7
			prostrate		9		
5.	5.	29	Leaf: intensity of green colour <u>without vernalization</u>				
			QN	VS A/ VG B	light		3
					medium	Conni	5
					dark	Limousine	7
			very dark	Limerick	9		

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	6.	30-35	Plant: growth habit <u>after</u> vernalization		
(+)	(*)	VS A/ VG B	erect		1
QN			semi-erect	Danube	3
			intermediate	Compact	5
			semi-prostrate	Baron	7
			prostrate		9
7.	7.	30-35	Leaf: intensity of green colour <u>after</u> vernalization		
	(*)	VS A/ VG B	light	Oxford	3
QN			medium	Compact	5
			dark	Bariris	7
			very dark	Rhythm	9
8.	8.	50	Plant: time of inflorescence emergence		
(+)	(*)	MS A/ MG B	very early	Adam 1	1
QN			early	Balin	3
			medium	Compact	5
			late	Baron	7
			very late	Hifi	9
9.	9.	50-58	Flag leaf: length		
(+)	(*)	MS A	short	Miracle	3
QN			medium	Compact	5
			long	Balin	7
10.	10.	50-58	Flag leaf: width		
(+)	(*)	MS A	narrow	Limousine	1
QN			medium	Compact	2
			broad	Lato	3

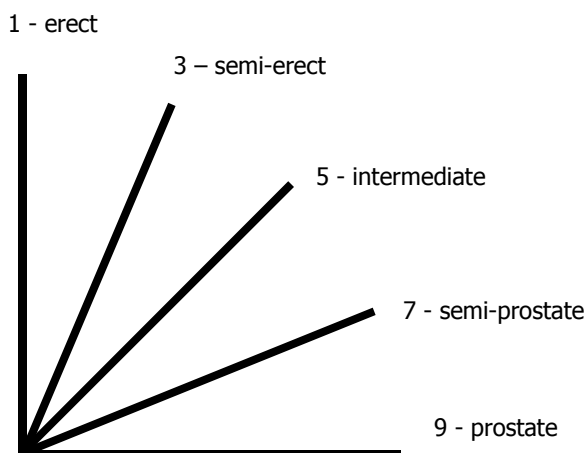
CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
11.	11.	58	Plant: natural height <u>when fully expanded</u>		
(+)	(*)	MS A/ MG B	short	Limousine	3
QN			medium	Compact	5
			tall	Likollo	7
			very tall	Lato	9
12.	12.	60-64	Inflorescence: shape of rachis		
(+)		VG A	straight	Compact	1
QL			bent	Baron	2
13.	13.	60-64	Inflorescence: form of collar of rachis		
(+)		VG A	closed	Compact	1
QL			open	Baron	2
14.	14.	64-68	Stem: length of upper internode		
(+)	(*)	MS A	short	Limousine	3
QN			medium	Compact	5
			long	Balin	7
15.	15.	64-68	Inflorescence: length		
(+)	(*)	MS A	short	Conni	3
QN			medium	Compact	5
			long	Balin	7
			very long	Lato	9
16.	16.	64-68	Inflorescence: anthocyanin coloration		
QN		VG A/ VG B	weak	Compact	3
			medium	Conni	5
			strong	Baron	7

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations for individual characteristics

Ad. 4: Plant: growth habit without vernalization

Ad. 6: Plant: growth habit after vernalization



Ad. 8: Plant: time of inflorescence emergence

The trial should be observed at least twice a week.

Plots with spaced plants:

The date of inflorescence emergence of each single plant should be assessed. A single plant is considered to have reached inflorescence emergence when the tip of three inflorescences can be seen protruding from the flag leaf sheath (just after DC 50). From the single plant data a mean date per plot and a mean date per variety are obtained.

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)

Ad. 9: Flag leaf: length

Ad. 10: Flag leaf: width

The Flag leaf is the first leaf below the inflorescence. Measurements should be made on the same leaf.

Length should be measured from the tip of the leaf blade to the leaf sheath.

Width should be measured at the widest point of the leaf blade.

Ad. 11: Plant: natural height when fully expanded

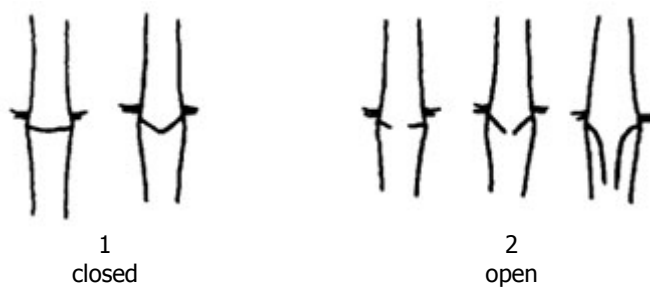
Measurements should be made in the field from ground level to the top of the plant, when the inflorescences are fully expanded.

Ad. 12: Inflorescence: shape of rachis

The shape of rachis should be observed opposite to the lower side branches.



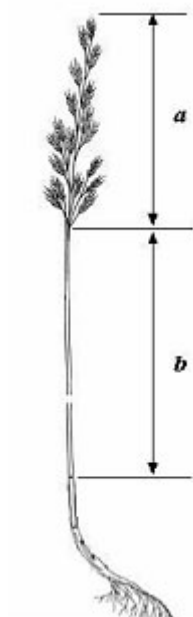
Ad. 13: Inflorescence: form of collar of rachis



Ad. 14: Stem: length of upper internode

Ad. 15: Inflorescence: length

Observations should be made on the longest stem. The upper internode (b) is the part of the stem above the upper node up to the beginning of the inflorescence (a).



8.2 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

9. LITERATURE

Meier, U., 1997: Growth stages of mono- and dicotyledonous plants. BBCH-Monograph Blackwell Science. Berlin, Vienna, a.o., 622 pp.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974: A decimal code for the growth stages of cereals. Weed Research 14: pp. 415 – 421.

10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/033/1