

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Secale cereale L.

RYE

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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 Secale cereale 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), the General Introduction DUS (UPOV Document such as to http://www.upov.int/export/sites/upov/resource/en/tg 1 3.pdf), its associated **TGP** documents (http://www.upov.int/tqp/en/) and the relevant UPOV Test Guideline TG/58/7 dated 17/12/2020 (https://www.upov.int/test_guidelines/en/fulltext_tgdocs.jsp?q=secale_) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **27.04.2022**. 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.

In case the variety is an agricultural hybrid, the variety descriptions of the parental components shall be added as well.

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 <u>Informing on problems in the DUS test</u>

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 pertinent agreement, on matters of particular urgency, 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 <u>Sample keeping in case of problems</u>

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 https://public.plantvarieties.eu/publication in the special issue S2/S3 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 vigor, 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.

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/edocs/tgpdocs/en/tgp 9.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 8.3

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: drilled plots
- C: special test

For the characteristics indicated by A, in case of inbred lines and single crosses from inbred lines, uniformity should be assessed on drilled plots (see chapter 4.2)

3.4 Test design

3.4.1 Open pollinated varieties, hybrid varieties and synthetic varieties: Each test should be designed to result in a total of at least 60 single spaced plants (A), which should be divided between at least 2 replicates. In addition, the test should include at least 300 plants in a drilled plot (B).

<u>Inbred lines and single crosses from inbred lines</u>: Each test should be designed to result in a total of at least 20 single spaced plants (A). In addition, the test should include at least 600 plants in drilled plots which should be divided between at least 2 replicates (B).

The assessment of the characteristic "seasonal type" should be designed to result in a total of at least 300 plants.

In case of hybrids, the parent lines have to be included in the test and should be tested and assessed as any other variety.

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 Special tests for additional characteristics

In accordance with Article 23 of Implementing Rules N° 874/2009 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 characteristics 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 FO.

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

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.

The variety collection shall include all varieties used as components (generally inbred lines) of all the hybrid varieties included in the variety collection, as well as varieties of common knowledge in their own right.

3.6.5 <u>Maintenance and renewal/update of a living variety collection</u>

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/edocs/tgpdocs/en/tgp-9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

To assess distinctness of hybrids, a pre-screening system on the basis of the parental lines and the formula may be established according to the following recommendations:

- (i) description of parental lines according to the Technical Protocols;
- (ii) check of the distinctness of the parental lines in comparison with the reference collection, based on the characteristics in the table of characteristics in order to screen the closest inbred lines;
- (iii) check of the distinctness of the hybrid formula in comparison with those of the hybrids in common knowledge, taking into account the closest inbred lines;
- (iv) assessment of the distinctness at the hybrid level of varieties with a similar formula.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

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.

If distinctness is assessed using the 2 x 1% criterion, the difference between two varieties is clear if the respective characteristics are significantly different in the same direction at the 1% level in at least two out of three years. 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.

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 significance level or statistical methods prescribed are not appropriate the method used should be clearly described.

4.1.4 Number of plants/parts of plants to be examined

Open pollinated varieties, hybrid varieties (excluding single crosses from inbred lines) and synthetic varieties: Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 60 plants or parts of plants taken from each of 60 plants and any other observations made on all plants in the test. In the case of observations of parts of plants, the number of parts to be taken from each of the plants should be 1.

<u>Inbred lines and single crosses from inbred lines:</u> Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts of plants taken from each of 10 plants and any other observations made on all plants in the test, disregarding any off-type plants. In the case of observations of parts of 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, observations on a group of plants (MG, VG) always refers to inbred lines and single crosses from inbred lines and observations on single plants (MS, VS) refers to open pollinated varieties, hybrid varieties and synthetic varieties.

4.2 Uniformity

- 4.2.1 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 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:
- 4.2.2 This Technical Protocol has been developed for the examination of open pollinated varieties, hybrid varieties, synthetic varieties, inbred lines and single crosses from inbred lines. For varieties with other types of propagation the recommendations in the UPOV-General Introduction to DUS 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 open pollinated varieties, hybrid varieties other than single crosses from inbred lines and synthetic varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.
- 4.2.4 For the assessment of uniformity of inbred lines and single crosses from inbred lines, the following standards should be applied:
 - For the assessment of uniformity in a sample size of 600 plants, a population standard of 0.5% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 600 plants, 6 off-types are allowed. For the assessment of uniformity in a sample size of 60 or 100 plants or parts of plants, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 100 plants or parts of plants, 5 off-types are allowed. In the case of a sample size of 60 plants, 3 off-types are allowed.
- 4.2.5 Where the assessment of a hybrid variety involves the parent lines, the uniformity of the hybrid variety should, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity of its parent lines.
- 4.2.6 For inbred lines and single crosses from inbred lines, a re-submission of plant material may be allowed for the second growing cycle if in the first growing cycle the number of off-types did not exceed 10 plants in a sample size of 600 (Population standard of 1 % with an acceptance probability of at least 95 %) or 10 plants or parts of plants in a sample size of 100 (Population standard of 6 % with an acceptance probability of at least 95 %).

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/edocs/tgpdocs/en/tgp 11.pd).

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 stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.
- 4.3.3 Where appropriate, or in cases of doubt, the stability of a hybrid variety may, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity and stability of its parent lines.

5. GROUPING OF VARIETIES AND ORGANISATION 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 organise the growing trial so that similar varieties are grouped together.
- **5.3** The following have been agreed as useful grouping characteristics:
 - a) Grain: intensity of colour of aleurone layer (characteristic 1)
 - b) Seasonal type (characteristic 21)
- **5.4** If other characteristics than those from the Technical Protocol 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.
- **5.5** Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

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.

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

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

6.3 Example Varieties

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

6.4 Legend

For column 'CPVO No':

G Grouping characteristic - see Chapter 5

QL Qualitative characteristic
QN Quantitative characteristic
PO Pseudo-qualitative characteristic

(+) Explanations for individual characteristics - see Chapter 8.2

For column 'UPOV No':

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

(*) UPOV Asterisked characteristic - Characteristics that are important for the international harmonization of variety descriptions.

For column `Stage, method': MG, MS, VG, VS

- see Chapter 4.1.5

(a) 00-99 Explanations covering several Characteristics - see Chapter 8.1 Explanations on growth stages - see Chapter 8.3 A, B, C - see Chapter 3.3

The example varieties are indicated as follows:

- (s) spring rye (w) winter rye

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1. (+)	1. (*)	VG C	Grain: intensity of colour of aleurone layer		
QL		00	light	(w) Helltop	1
G			dark	(s) Arantes, (w) Bonfire	2
2. (+)	2.	VG C	Grain: coloration with phenol		
QN		00	absent or very light		1
			very light to light		2
			light		3
			light to medium		4
			medium	(s) Tiroler, (w) Gonello	5
			medium to dark		6
			dark	(s) Arantes, (w) Marcelo	7
			dark to very dark		8
			very dark	(w) SU Stakkato	9
3. (+)	3. (*)	VG C	Coleoptile: anthocyanin coloration		
QN		10-11	absent or very weak	(w) Helltop	1
			very weak to weak		2
			weak		3
			weak to medium		4
			medium	(w) Tonus	5
			medium to strong		6
			strong	(s) Ovid, (w) Turbogreen	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
4.	4.	MS C	Coleoptile: length		
QN		12-13	very short		1
		(a)	very short to short		2
			short	(w) Dukato	3
			short to medium		4
			medium	(s) Arantes, (w) Marcelo	5
			medium to long		6
			long	(w) Higreen	7
			long to very long		8
			very long		9
5.	5.	MS C	First leaf: length of sheath		
QN		12-13	very short		1
		(a)	very short to short		2
			short		3
			short to medium		4
			medium	(s) Arantes, (w) Marcelo	5
			medium to long		6
			long	(w) Jobaro	7
			long to very long		8
			very long		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	6.	MS C	First leaf: length of blade		
QN		12-13	very short		1
		(a)	very short to short		2
			short	(w) Guttino	3
			short to medium		4
			medium	(w) Marcelo	5
			medium to long		6
			long	(w) Turbogreen	7
			long to very long		8
			very long		9
7. (+)	7. (*)	VG A/ VG B/ VS A	Plant: growth habit		
QN		25-29	erect		1
			erect to semi-erect		2
			semi-erect		3
			semi-erect to intermediate		4
			intermediate	(s) Tiroler, (w) Turbogreen	5
			intermediate to semi-prostrate		6
			semi-prostrate	(w) Guttino	7
			semi-prostrate to prostrate		8
			prostrate		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
8. (+)	8. (*)	MG A/ MG B/ MS A	Time of ear emergence		
QN			very early	(w) Bonfire	1
			very early to early		2
			early	(w) Turbogreen	3
			early to medium		4
			medium	(w) Jobaro	5
			medium to late		6
			late		7
			late to very late		8
			very late	(w) Tonus	9
9. (+)	9. (*)	VG B	Flag leaf: glaucosity of sheath		
QN		54/58	absent or very weak		1
			very weak or weak		2
			weak	(w) Bonfire	3
			weak to medium		4
			medium	(w) Helltop	5
			medium to strong		6
			strong	(w) SU Stakkato	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
10.	10.	MS A	Penultimate leaf: length of blade		
QN		60-69	very short		1
			very short to short		2
			short	(w) Guttino	3
			short to medium		4
			medium	(w) Helltop	5
			medium to long		6
			long	(w) Turbogreen	7
			long to very long		8
			very long		9
11.	11.	MS A	Penultimate leaf: width of blade		
QN		60-69	very narrow		1
			very narrow to narrow		2
			narrow	(w) Tonus	3
			narrow to medium		4
			medium	(w) Marcelo	5
			medium to broad		6
			broad	(w) Virgiai	7
			broad to very broad		8
			very broad		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12.	12. (*)	VG A/ VG B/ VS A	Ear: glaucosity		
QN		69-75	absent or very weak		1
			very weak or weak		2
			weak	(w) Tonus	3
			weak to medium		4
			medium	(s) Tiroler, (w) Marcelo	5
			medium to strong		6
			strong	(w) SU Stakkato	7
			strong to very strong		8
			very strong		9
13. (+)	13. (*)	VG A/ VG B/ VS A	Stem: density of hairs below ear		
QN		70-85	absent or very sparse		1
			very sparse or sparse		2
			sparse	(w) Guttino	3
			sparse to medium		4
			medium	(w) Tonus	5
			medium to dense		6
			dense	(w) KWS Dolaro	7
			dense to very dense		8
			very dense		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
14. (+)	14. (*)	MS A	Plant: length		
QN		80-92	very short		1
			very short to short		2
			short	(w) Guttino	3
			short to medium		4
			medium	(s) Ovid, (w) Marcelo	5
			medium to long		6
			long	(w) Jobaro	7
			long to very long		8
			very long	(w) Bonfire	9
15.	15.	MS A	Stem: length between upper node and ear		
QN		80-92	very short		1
			very short to short		2
			short	(w) KWS Dolaro	3
			short to medium		4
			medium	(w) Marcelo	5
			medium to long		6
			long	(w) Tonus	7
			long to very long		8
			very long	(w) Turbogreen	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
16. (+)	16. (*)	MS A	Ear: length		
QN		80-92	very short		1
			very short to short		2
			short	(s) Arantes, (w) Imperator	3
			short to medium		4
			medium	(w) Turbogreen	5
			medium to long		6
			long	(s) Tiroler, (w) Tonus	7
			long to very long		8
			very long		9
17. (+)	17. (*)	MS A	Ear: density		
QN		80-92	very lax		1
			very lax to lax		2
			lax	(w) Bonfire	3
			lax to medium		4
			medium	(s) Ovid, (w) Gonello	5
			medium to dense		6
			dense	(w) Helltop	7
			dense to very dense		8
			very dense		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
18. (+)	18.	VG A/ VG B/ VS A	Ear: attitude		
QN		90-92	erect		1
			erect to semi-erect		2
			semi-erect		3
			semi-erect to horizontal		4
			horizontal	(w) Terogrün	5
			horizontal to semi-recurved		6
			semi-recurved	(w) Helltop	7
			semi-recurved to recurved		8
			recurved		9
19. (+)	19. (*)	MG	Grain: thousand grain weight		
QN		92	very small		1
			very small to small		2
			small	(w) Tonus	3
			small to medium		4
			medium	(w) Turbogreen	5
			medium to large		6
			large	(w) Jobaro	7
			large to very large		8
			very large		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
20. (+)	20. (*)	MG	Grain: length		
QN		92	very short		1
			very short to short		2
			short	(w) Tonus	3
			short to medium		4
			medium	(s) Arantes, (w) Gonello	5
			medium to long		6
			long	(w) Jobaro	7
			long to very long		8
			very long		9
21. (+)	21. (*)	VG	Seasonal type		
PQ			winter	(w) SU Stakkato	1
			alternative		2
G			spring	(s) Arantes	3

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

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

a) Seeds are sown in multipot plates with standard soil in 1 cm sowing depth. The plants are produced in the greenhouse at 20°C and with additional light for 12 hours per day for 12 days. The test should be designed to result in a total of at least 60 plants.

8.2 Explanations for individual characteristics

Ad. 1: Grain: intensity of colour of aleurone layer

The observations should be made on a sample of 100 seeds.

Ad. 2: Grain: coloration with phenol

Number of grains per test: 100

The grains should not have been treated chemically

Preparation of grains: Soak in tap water for 16 to 20 hours, drain and remove surface water, place the

grains with crease downwards, cover dish with lid

Concentration of solution: 1% Phenol-solution (freshly made up)
Around of solution: 2 ml in a petri-dish on filter paper

Place: Laboratory

Light: Daylight, out of direct sunshine

Temperature: 18 to 20 °C

Time of recording: 4 hours after adding solution

Scale of recording See chapter 7. Tale of Characteristics

Note: At least two of the example varieties should be included as a control

Ad. 3: Coleoptile: anthocyanin coloration

Number of seeds per test: 100

Preparation of seeds: Set up non-dormant seeds on moistened filter paper covered with Petri dish lid

during germination

Place: Laboratory or greenhouse

Temperature and light: When the coleoptiles have reached length of about 1 cm at 15 to 16°C in the dark,

they are placed in continuous light (daylight equivalent) of 13000 to 15000 lux at

18 to 19°C for 4 days

Time of recording: Coleoptiles fully developed, growth stage 09-11

Note: At least two example varieties should be included as a control

Any alternative method may be used if it gives the same results.

Ad. 7: Plant: growth habit



Ad. 8: Time of ear emergence

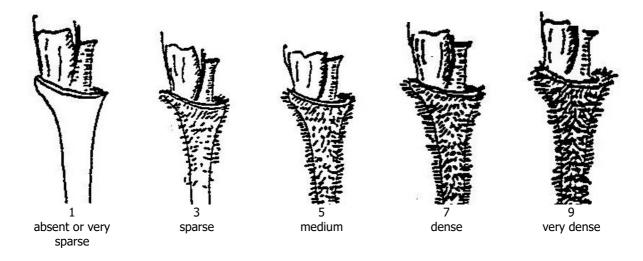
<u>Open pollinated varieties, hybrid varieties and synthetic varieties (MS/A)</u>: The number of plants which have reached growth stage 52 should be recorded at two-day intervals. From this data, the average time of ear emergence of the variety should be calculated.

<u>Inbred lines and single crosses from inbred lines (MG/A, MG/B):</u> Time of ear emergence is reached when 50% of the plants have reached growth stage 52.

Ad. 9: Flag leaf: glaucosity of sheath

The observation should be done on the upper third of the sheath.

Ad. 13: Stem: density of hairs below ear



Ad. 14: Plant: length

Pant length should be measured including stem, ear, and awns.

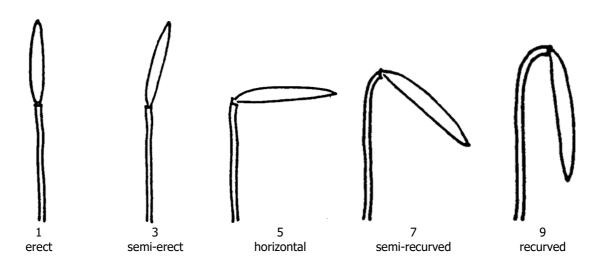
Ad. 16: Ear: length

Ear length should be measured without awns.

Ad. 17: Ear: density

Ear density is the number of rachis segments divided by length of ear.

Ad. 18: Ear: attitude



Ad. 19: Grain: thousand grain weight

Ad. 20: Grain: length

Thousand grain weight and grain length should be assessed in a harvested bunch.

Ad. 21: Seasonal type

The seasonal type (need of vernalization) should be assessed on plots sown in springtime. Example varieties should always be included in the trial. When the example varieties behave according to their descriptions, the varieties under study can be described. At the time when the latest spring type variety is fully mature (stage 91-92 of the Zadoks decimal code) the growth stage reached by the respective variety should be assessed. The states of expression are defined as follows:

- 1 Winter type (high need of vernalization): The plants have reached stage 45 of the Zadoks decimal code (boots swollen) at maximum.
- 2 Alternative type (partial need of vernalization): The plants have exceeded stage 45 of the Zadoks decimal code (they should normally have exceeded stage 75) and have reached stage 90 at maximum.
- 3 Spring type (no need or very weak need of vernalization): The plants have exceeded stage 90 of the Zadoks decimal code.

8.3 Explanations on growth stages

Descriptions of the growth stages of the Zadocks decimal codes for cereals (ZADOCKS et al., 1974)

Zadocks Decimal code	Description	Zadocks Decimal code	Description
	Germination		Inflorescence emergence
00	Dry seed		
01	Start of imbibition	51	First spikelet of inflorescence visible
03	Imbibition complete	52	-
05	Radicle emerged from seed	53	1/4 of inflorescence emerged
07	Coleoptile emerged from seed	54	
09	Leaf just at coleoptile tip	55 57 58	1/2 of inflorescence emerged 3/4 of inflorescence emerged -
	Seedling growth	59	Emergence of inflorescence completed
10	First leaf through coleoptile		completed
11	First leaf unfolded		
12	2 leaves unfolded		
13	3 leaves unfolded		<u>Anthesis</u>
14	4 leaves unfolded	60	-
15	5 leaves unfolded	61	Beginning of anthesis
16	6 leaves unfolded	65	Anthesis half-way
17	7 leaves unfolded	69	Anthesis completed
18	8 leaves unfolded	05	7 that is is completed
19	9 or more leaves unfolded		
	Tillering		Milk development
20	Main shot only	70	
21	Main shot only and 1 tiller	71	Caryopsis watery ripe
22	Main shot only and 2 tillers	73	Early milk
23	Main shot only and 3 tillers	75 77	Medium milk
24	Main shot only and 4 tillers	77	Late milk
25 26	Main shot only and 5 tillers		
27	Main shot only and 6 tillers		
28	Main shot only and 7 tillers Main shot only and 8 tillers		Dough development
29	Main shot only and 9 or more tillers	80	<u>Dough development</u>
29	Main shot only and 9 of more tillers	83	Early dough
	Stem elongation	85	Soft dough
30	Pseudo stem erection	87	Hard dough
31	1 st node detectable	07	riaru uougii
32	2 nd node detectable		
33	3 rd node detectable		
34	4 th node detectable		Ripening
35	5 th node detectable	91	Caryopsis hard (difficult to divide with thumbnail)
36	6 th node detectable	92	Caryopsis hard (no longer dented with thumbnail)
37	Flag leaf just visible	93	Caryopsis loosening in daytime
39	Flag leaf ligule/collar just visible	94 95	Overripe, straw dead and collapsing Seed dormant
	<u>Bolting</u>	96	Viable seed giving 50% germination
41	Flag leaf sheath extending	97	Seed not dormant
43	Boots just visibly swollen	98	Secondary dormancy induced
45	Boots swollen	99	Secondary dormancy lost
47	Flag leaf sheath opening		
49	First awn visible		

9. LITERATURE

ZADOKS, J. C., CHANG, T. T. and KONZAK, C. F., 1974. A decimal code for the growth stages of cereals. Weed Research, 14: 415-421

10. **TECHNICAL QUESTIONNAIRE**

The Technical Questionnaire is available on the $\underline{\text{CPVO website}}$ under the following reference: CPVO-TQ/058/1-Rev - Secale cerale L. - rye

Link to e-TQ:
Technical Questionnaires | CPVO (europa.eu)