

# PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Triticum turgidum L. subsp. durum (Desf.) Husn.

## **DURUM WHEAT**

UPOV Code: TRITI\_TUR\_DUR

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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 *Triticum turgidum* L. subsp. durum (Desf.) Husn.

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 <a href="http://www.upov.int/export/sites/upov/resource/en/tg\_1\_3.pdf">http://www.upov.int/export/sites/upov/resource/en/tg\_1\_3.pdf</a>), its associated TGP documents (<a href="http://www.upov.int/tgp/en/">http://www.upov.int/tgp/en/</a>) and the relevant UPOV Test Guideline TG/120/3 dated 28/03/2012 (<a href="http://www.upov.int/edocs/tgdocs/en/tg120.pdf">http://www.upov.int/edocs/tgdocs/en/tg120.pdf</a>) for the conduct of tests for Distinctness, Uniformity and Stability.

### 1.2 Entry into Force

The present protocol enters into force on **01/03/2014**. Any on-going 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 <a href="http://cpvo.europa.eu/applications-and-examinations/technical-examinations/submission-of-plant-material-s2-publication">http://cpvo.europa.eu/applications-and-examinations/submission-of-plant-material-s2-publication</a> 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.

## 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" <a href="http://www.upov.int/edocs/tapdocs/en/tap-9.pdf">http://www.upov.int/edocs/tapdocs/en/tap-9.pdf</a>.

#### 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

### 3.4 Test design

Each test should be designed to result in a total of at least 2000 plants, which should be divided between at least two replicates.

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

If ear rows are used, the test should be conducted on at least 100 ear rows.

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 examination office unless special cooperation exists between examination offices and the CPVO. The descriptive and pictorial information produced by the examination office shall be held and maintained in a form of a database

#### 3.6.2 Living Plant Material

The examination office 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 examination office.

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

The examination office 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'  $\frac{\text{http://www.upov.int/edocs/tgpdocs/en/tgp 9.pdf}}{\text{http://www.upov.int/edocs/tgpdocs/en/tgp 9.pdf}}) \ prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.}$ 

#### 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  $2 \times 1\%$  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-tiled 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 proposed are not appropriate the method used should be clearly described.

### 4.1.4 Number of plants/parts of plants to be examined

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."

#### 4.1.5 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' (<a href="http://www.upov.int/edocs/tgpdocs/en/tgp">http://www.upov.int/edocs/tgpdocs/en/tgp</a> 10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

For the assessment of uniformity in a sample of 2000 plants, a population standard of 0.1 % and an acceptance probability of at least 95 % should be applied. In the case of a sample size of 2000 plants, 5 off-types are allowed.

For the assessment of uniformity in a sample of 100 ear-rows, plants or parts of plants, a population standard of 1 % and an acceptance probability of at least 95 % should be applied. In the case of a sample size of 100 ear-rows, plants or parts of plants, 3 off-types are allowed.

An ear-row is considered to be an off-type ear-row if there is more than 1 off-type plant within that ear-row.

The recommended sample size for the assessment of uniformity is indicated by the following key in the table of characteristics:

- {A} sample size of 100
- {B} sample size of 2000

For characteristics with the key "A" in the list of characteristics the assessment of uniformity can be done in 2 steps. In a first step, 20 plants or parts of plants are observed. If no off-types are observed, the variety is declared to be uniform. If more than 3 off-types are observed, the variety is declared not to be uniform. If 1 to 3 off-types are observed, an additional sample of 80 plants or parts of plants must be observed.

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 15 plants in a sample size of 2000 plants (Population standard of 0.5% with an acceptance probability of  $\geq$  95%) or 9 plants, parts of plants or ear rows in a sample size of 100 (Population standard of 5% with an acceptance probability of  $\geq$  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' (<a href="http://www.upov.int/edocs/tgpdocs/en/tgp">http://www.upov.int/edocs/tgpdocs/en/tgp</a> 11.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.

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.

#### 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) Grain: coloration with phenol (characteristic 1)
  - (b) Lower glume: hairiness of external surface (characteristic 19)
  - (c) Straw: pith in cross section (characteristic 20)
  - (d) Awn: colour (characteristic 21)
  - (e) Ear: coloration (characteristic 23)
  - (f) Plant: seasonal type (characteristic 27)
- **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 examination office 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

G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	– see Chapter 6.1.2 in UPOV guideline
MG, MS, VG, V	/S	– see Chapter 4.1.5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
Α	Sample size 100	- see Chapter 4.2
В	Sample size 2000	- see Chapter 4.2
(a)	See Explanations on the Table of Charac	cteristics in Chapter 8.1
(+)	See Explanations on the Table of Charac	cteristics in Chapter 8.2
00 – 99	See Explanations on the Table of Charac	cteristics in Chapter 8.3

## 7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples	Note
1.		00	Seed: coloration with phenol		
(+)		VG	absent or very light	Lupidur (W), Qualidou	1
QN			light	Kiradur (W), Iride	3
G		A	medium	Italo	5
			dark	Matt	7
			very dark	Donduro	9
2.	1.	09-11	Coleoptile: anthocyanin coloration		
(+)		VG	absent or very weak	Ciccio	1
QN			weak	Arcangelo	3
		A	medium	Don Jaime	5
			strong	Iride	7
			very strong	Don Ricardo	9
3.	2.	25-29	Plant: growth habit		
(+)	(*)	VG	erect	-	1
QN		В	semi-erect	Actisur	3
			intermediate	Don Sebastian	5
			semi prostrate	Cliodur (W), Soldur	7
			prostrate	-	9
4.	3.	50-51	Frequency of plants with recurved flag leaves		
(+)		VG	absent or very low	Colosseo	1
QN		В	low	Don Jose	3
			medium	Don Jaime	5
			high	Orobel	7
			very high	-	9

The optimum stage of development as well as method of observation for the assessment of each characteristic is indicated by numbers and letters. Explanations are given in Annex 1 in 'Explanations and Methods'.

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples	Note
5.	4.	50-51	Time of ear emergence		
(+)	(*)	MG	very early	Ismur	1
			early	Superdur (W), Cantico	3
QN		В	medium	Kiradur (W), Iride	5
			late	Creso	7
6	5.	55-59	Flag leaf: anthocyanin coloration of auricles		
QN		VG	absent or very weak	Don Pedro	1
		В	weak	Carpio, Amilcar	2
			medium	Don Jose, Don Ricardo	3
			strong	Carioca, Arcobaleno	4
			very strong	Tiziana	5
7.	6.	55-65	Flag leaf: glaucosity of sheath		
QN	(*)	VG	absent or very weak	Meridiano	1
		В	weak	-	3
			medium	Aceres	5
			strong	Don Sebastian	7
			very strong	Kiradur (W), Colosseo	9
8.	7.	55-65	Flag leaf: glaucosity of lower side of leaf blade		
QN	(*)	VG	absent of very weak	Aventur	1
		В	weak	Duilio	3
			medium	Colosseo	5
			strong	Elsadur (W), Chiara	7

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples	Note
9.	8.	55-69	Culm: density of hairiness of uppermost node		
(+)		VG	absent or very weak	Lupidur (W), Don Sebastian	1
QN		A	weak	Kiradur (W), Canyon	3
			medium	Arnacoris	5
			strong	Superdur (W), Levante	7
			very strong	-	9
10.	9.	60-69	Culm: glaucosity of neck		
QN	(*)	VG	absent or very weak	Meridiano	1
		В	weak	-	3
			medium	Don Jose	5
			strong	Elsadur (W), Don Sebastian	7
			very strong	Sy Lido	9
11.	10.	60-69	Ear: glaucosity		
QN	(*)	VG	absent or very weak	Ofanto	1
		В	weak	Marco	3
			medium	Don Jose	5
			strong	Don Sebastian	7
			very strong	Atoudur	9
12.	11.	75-92	Plant: length		
(+)	(*)	MG	very short	Arcangelo	1
QN		В	short	Ciccio	3
			medium	Don Jose	5
			long	Kiradur (W), Valbelice	7
13.	13.	75-92	Ear: length of awns at tip relative to length of ear		
(+)	(*)	VG	shorter	Plussur	1
QN		В	equal	Arcobaleno	2
			longer	Qualidou	3

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples	Note
14.	14.	80-92	Lower glume: shape		
(+)		VG	ovoid	Canyon	1
PQ		A	medium oblong	Don Jose	2
		(a)	narrow oblong	Don Sebastian	3
15.	15.	80-92	Lower glume: shape of shoulder		
(+)		VG	sloping	Colosseo	1
PQ		A	rounded	Iride	2
		(a)	straight	Don Jose	3
			elevated	Kiko Nick	4
			elevated with a 2 <sup>nd</sup> beak	Don Sebastian	5
16.	16.	80-92	Lower glume: width of shoulder		
(+)		VG	very narrow	Don Sebastian	1
QN		A	narrow	Lupidur (W), Asdrubal	3
		(a)	medium	Orobel	5
			broad	-	7
17.	17.	80-92	Lower glume: length of beak		
QN		VG	very short	Duilio	1
		A	short	Logidur (W), Pictur	3
		(a)	medium	Superdur (W), Don Jose	5
			long	Levante	7
			very long	Vetrodur	9
18.	18.	80-92	Lower glume: curvature of beak		
(+)		VG	absent	Simeto	1
QN		A	weak	Iride	3
			moderate	Don Jaime	5
			strong	Don Isidoro	7

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples	Note
19. (+)	19. (*)	80-92	Lower glume: hairiness of external surface		
QL		VG A	absent	Don Sebastian	1
G		(a)	present	Don Jose	9
20.	20.	90-92	Straw: pith in cross section		
(+)	(*)	VG	thin	Canyon	1
QN		A	medium	Arnacoris	2
G			thick	Lupidur (W), Chiara	3
21.	21.	90-92	Awn: colour		
PQ	(*)	VG	white	Lupidur (W), Don Sebastian	1
G		В	light brown	MV Makaroni (W), Duilio	2
			medium purple	Auradur (W), Grecale	3
			dark purple	Karur (W), Don Jose	4
22.	22.	90-92	Ear: length (excluding awns)		
QN	(*)	VG/MS	short	Don Jaime	3
		B/A	medium	Duilio	5
			long	Kiradur (W), Levante	7
23.	23.	90-92	Ear: coloration		
PQ	(*)	VG	white	Lupidur (W), Don Jose	1
G		В	slightly coloured	MV Makaroni (W)	2
			strongly coloured	GK Basa (W)	3
24.	24.	92	Ear: density		
(+)	(*)	VG/MS	lax	Levante	3
QN		B/A	medium	Elsadur (W), Iride	5
			dense	Auradur (W), Arcangelo	7
25.	25.	92	Grain: length of brush hair		
(+)	(*)	VG	short	Logidur (W), Arcangelo	1
QN		A	medium	Auradur (W), Carpio	3
			long	Kiradur (W), Orssur	5

CPVO N°	UPOV N°	Stage1 Method	Characteristics	Examples	Note
26.	26.	92	Grain: shape		
(+)		MS/VG	slightly elongated	Amilcar	1
QN		A	moderately elongated	Bolo	2
			strongly elongated	Creso	3
27.	28.	VG	Plant: seasonal type		
(+)			winter type	Lupidur (W), MV Makaroni (W)	1
PQ			alternative type	Simeto	2
G			spring type	Levante, Amilcar	3

#### 8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

## 8.1 Explanations covering several characteristics

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

(a) All observations on the spikelet should be made in the mid-third of the ear.

#### 8.2 Explanations for individual characteristics

#### Ad. 1: Seed: coloration with phenol

## Method for Determination of Phenol Reaction

Number of grains per test: 100 grains. The grains should not have been treated chemically

Equipment: Petri dishes (approx. 9 cm diameter).

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 per cent Phenol-solution (freshly made up)
Amount of solution: 1 per cent Phenol-solution (freshly made up)
The grains should be about 3/4 covered.

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 characteristic 1 in the Table of Characteristics

#### Ad 2: Coleoptile: anthocyanin coloration

#### Method for the Determination of Anthocyanin Coloration

Number of grains per test: 100 grains for distinctness and uniformity

Preparation of grains: Set up non-dormant grains on moistened filter paper with a Petri dish lid during

germination

Place: Laboratory or glasshouse

Light: After the coleoptiles have reached a length of about 1 cm in darkness, they are

placed in artificial light (daylight equivalent), 12,000 to 15,000 lux continuously

for 3 - 4 days

Temperature: 15 to 20<sup>o</sup>C.

Time of recording: Coleoptiles fully developed (about 1 week) at stage 09-11

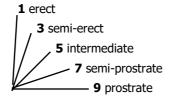
Scale of recording: See characteristic 1 in the Table of Characteristics

Note: Any other method used than the above mentioned must prove to provide the

same results.

## Ad 3: Plant: growth habit

The growth habit should be assessed visually from the attitude of the leaves and tillers at tillering stage (growth stages 25-29). The angle formed by the outer leaves and the tillers with an imaginary middle axis should be used.



The growth habit should be assessed visually from the attitude of the leaves and tillers. The angle formed by the outer leaves and the tillers with an imaginary vertical axis should be used.

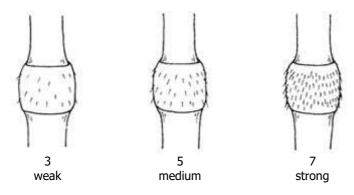
## Ad 4: Frequency of plants with recurved flag leaves

- 1.....all flag leaves are rectilinear
- 3..... about 1/4 of the plants with recurved flag leaves
- 5.....about 1/2 of the plants with recurved flag leaves
- 7.....about 3/4 of the plants with recurved flag leaves
- 9.....all flag leaves are recurved

## Ad 5: Time of ear emergence

The time of ear emergence is reached when the first spikelet is visible on ears of 50% of the plants.

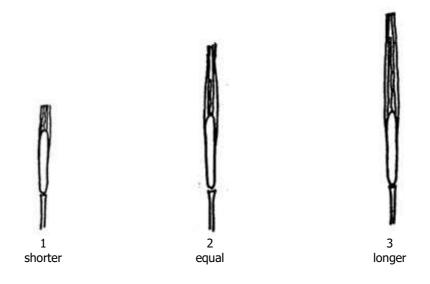
## Ad 9: Culm: density of hairiness of uppermost node



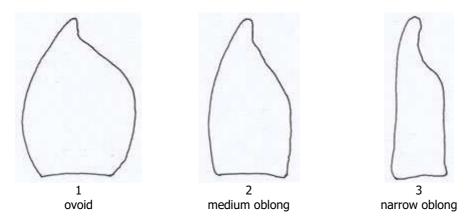
## Ad 12: Plant: length

Plant length should be measured including stem, ear and awn. The length is taken from the base of the plant to the tip of the highest awn.

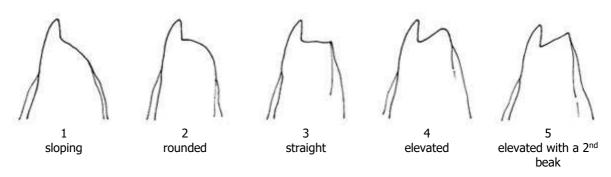
## Ad 13: Ear: length of awns at tip relative to length of ear



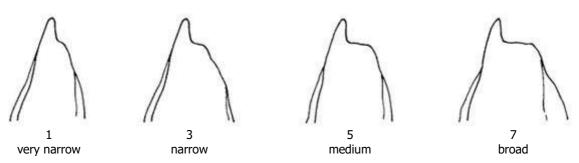
Ad 14: lower glume: shape



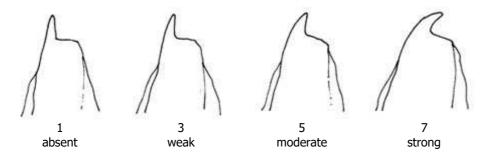
Ad 15: Lower glume: shape of shoulder



Ad 16: Lower glume: width of shoulder



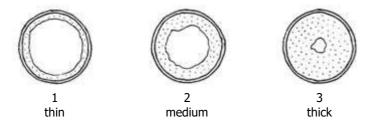
Ad 18: Lower glume: curvature of beak



Ad 19: Lower glume: hairiness of external surface

Observations should be made with a hand lens (x10 magnification).

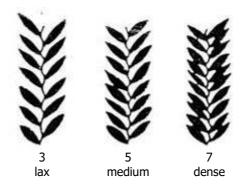
## Ad 20: Straw: pith in cross section



The pith in cross section should be observed half way between base of ear and stem node below. All stems of the plant should be checked and the strongest expression per plant recorded.

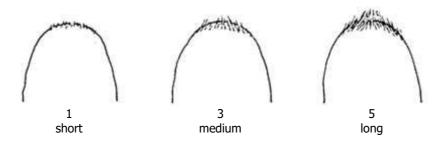
## Ad 24: Ear: density

Ear density can be determined by counting the number of spikelets and then dividing the number by the ear length. The higher ratio will indicate a higher density.



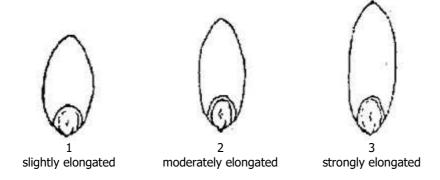
## Ad 25: Grain: length of brush hair

Observations should be made with a hand lens (x10 magnification). Brush hair length is viewed from the top of the grain on the dorsal side and can be described in the following ways:



#### Ad 26: Grain: shape

To be observed in dorsal view.



#### Ad 27: Plant: seasonal type

The seasonal type (need for vernalization) should be assessed on one or several plots sown in springtime. At the time when the latest spring type variety is fully mature (growth 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:

Winter type: The plants have not exceeded stage 45 of the Zadoks decimal code (boots swollen).

Alternative type: The plants have exceeded stage 45 of the Zadoks decimal code -- as a rule they have

exceeded stage 75 -- and have not exceeded stage 90.

Spring type: The plants have exceeded stage 90 of the Zadoks decimal code.

## 8.3 Growth stages

# The descriptions of the growth stages of the Zadoks decimal code for cereals (Zadoks et al., 1974)

Zadoks Decimal	Description
code 00	Dry seed
01	Start of imbibition
03	Imbibition complete
05	Radicle emerged from seed
07	Coleoptile emerged from seed
09	Leaf just at coleoptile tip
10	First leaf through coleoptile
11	First leaf unfolded
12	2 leaves unfolded
13	3 leaves unfolded
14	4 leaves unfolded
15	5 leaves unfolded
16	6 leaves unfolded
17	7 leaves unfolded
18	8 leaves unfolded
19	9 or more leaves unfolded
20	Main shoot only
21	Main shoot and 1 tiller
22	Main shoot and 2 tillers
23	Main shoot and 3 tillers
24	Main shoot and 4 tillers
25	Main shoot and 5 tillers
26	Main shoot and 6 tillers
27	Main shoot and 7 tillers
28	Main shoot and 8 tillers
29	Main shoot and 9 or more tillers
30	Pseudo stem erection
31	1st node detectable
32	2nd node detectable
33	3rd node detectable
34	4th node detectable
35	5th node detectable
36	6th node detectable
37	Flag leaf just visible
39	Flag leaf ligule/collar just visible
40	-

Zadoks Decimal code	Description	
41	Flag leaf sheath extending	
45	Boots just swollen	
47	Flag leaf sheath opening	
49	First awns visible	
50	First spikelet of inflorescence visible	
53	1/4 of inflorescence emerged	
55	1/2 of inflorescence emerged	
57	3/4 of inflorescence emerged	
59	Emergence of inflorescence completed	
60	Beginning on anthesis	
65	Anthesis half-way	
69	Anthesis completed	
70	-	
71	Kernel watery ripe	
73	Early milk	
75	Medium milk	
77	Late milk	
80	-	
83	Early dough	
85	Soft dough	
87	Hard dough	
90	-	
91	Kernel hard (difficult to divide with thumbnail)	
92	Kernel hard (no longer dented with thumbnail)	
93	Kernel loosening in daytime	
94	Overripe, straw dead and collapsing	
95	Seed dormant	
96	Viable seed giving 50% germination	
97	Seed not dormant	
98	Secondary dormancy induced	
99	Secondary dormancy lost	

## 9. LITERATURE

Annicchiarico, P., Pecetti, L., 1994: Morpho-physiological traits as descriptors for discrimination of durum wheat germplasm. Genetic Resources and Crop Evoluation. Kluwer Academic Publishers. NL, 41: 47-54.

Fitzsimmons, R.W., Martin, R.H., Roberts, G.I., Wrigley, C.W., 1986: Australian Cereal Identification. Commonwealth Scientific and Industrial Research Organization. East Melbourne, AU.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974: A Decimal Code for the Growth Stages of Cereals. Weed Research. NL, 14: 415-421.

Sparks, G.A., Bezar, H.J., Lamberrts, R., 1987: Identification of New Zealand Wheat Cultivars. Crop Research Division, DISR. Christchurch, NZ.

# 10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference: CPVO-TQ/120/3