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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

DURUM WHEAT

UPOV Code: TRITI_TUR_DUR

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

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by an expert from Australia

to be considered by the
Technical Working Party for Agricultural Crops at its thirty-eighth session,
to be held in Seoul, Republic of Korea, from August 31 to September 4, 2009

Alternative Names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Triticum turgidum subsp. durum (Desf.) Husn. *Triticum durum* Desf. *Triticum turgidum* subsp. *turgidum* conv. *durum* (Desf.) MacKey *Triticum turgidum* L.	Durum Wheat	Blé dur	Hartweizen	Trigo Duro

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

Other associated UPOV documents: TG/3/11 + Corr. Wheat

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Triticum turgidum* subsp. *durum* (Desf.) Husn.

2. Material Required

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

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

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

3 kg.

(5kg proposed by France)

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

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 Number of Growing Cycles

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

3.2 Testing Place

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

3.3 Conditions for Conducting the Examination

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

3.3.2 Stage of development for the assessment

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

3.3.3 Type of observation

The recommended method of observing the characteristic is indicated by the following key in the second column of the Table of Characteristics:

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

3.3.4 Type of plot for observation

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

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

3.3.5 Observation of color by eye

Because daylight varies, color determinations made against a color chart should be made either in a suitable cabinet providing artificial daylight or in the middle of the day in a room without direct sunlight. The spectral distribution of the illuminant for artificial daylight should conform with the CIE Standard of Preferred Daylight D 6500 and should fall within the tolerances set out in the British Standard 950, Part I. These determinations should be made with the plant part placed against a white background.

3.4 Test Design

3.4.1 Each test should be designed to result in a total of at least 2,000 plants, which should be divided between 2 or more replicates.

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

3.5 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, 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.

3.6 Additional Tests

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.2 Uniformity

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 For the assessment of uniformity, 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 2,000 plants, 5 off-types are allowed.

Comment from Portugal: there is no ear-row method to evaluate uniformity.

For a sample size of 100 ear-rows the number of off-types should not exceed 3 in 100. (population standard of 1% and an acceptance probability of at least 95 %).

4.3 Stability

4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

(a) Plant: time of ear emergence (first spikelet is visible on ears of 50% plants) (characteristic 4)

Comment from Austria: Ch4 is not useful for grouping.

(b) Plant: height (stem, ear and awn) (characteristic 13)

Comment from Austria: Ch13 is not useful for grouping.

(c) Ear: distribution of awns (characteristic 14)

Comment from Austria and France: Ch14 is not useful for grouping

(d) Lower glume: hairiness of external surface (characteristic 21)
(e) Straw: pith in cross section (half way between base of ear and stem node below) (characteristic 22)
(f) Ear: color at maturity (characteristic 25)
(g) Plant: seasonal type (characteristic 38)

Comment from France: Ch38 is not useful for grouping .

The following grouping characteristics are proposed by France:

(h) Awn: color (characteristic 23)

(i) Grain: coloration with phenol (characteristic 37)

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

6. Introduction to the Table of Characteristics

6.1 Categories of Characteristics

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 States of Expression and Corresponding Notes

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 Types of Expression

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 Example Varieties

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

6.5 Legend

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3
QN: Quantitative characteristic – see Chapter 6.3
PQ: Pseudo-qualitative characteristic – see Chapter 6.3

MG, MS, VG, VS: –see Chapter 3.3.3

A: spaced plants
B: row plot
C: special test
GS: growth stage.

(+) See Explanations on the Table of Characteristics in Chapter 8.2

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

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1. **(+)**	**GS 09-11 VG**	**Coleoptile: intensity of anthocyanin coloration**					
QN	**C**	absent or very weak				Fara, Kronos, Valgiorgio	1
		weak				Campomoro	3
		medium				Capdur, Chandur, Yallaroi	5
		strong				Kamilaroi, Primadur, Wollaroi	7
		very strong				EGA Bellaroi, Miradur, Tamaroi	9
2. **(+)**	**GS 10-11 VG**	**First leaf: intensity of anthocyanin coloration**					
QN	**C**	absent or very weak				Kronos	1
		weak				Tamaroi, Yallaroi	3
		medium				Cargivox	5
		strong				Enrico Avanzi	7
		very strong				Aldura	9
Comment: France has proposed to delete Characteristic 2. No difference between varieties - Difficult work on greenhouse.							
3. **(*)** **(+)**	**GS 25-29 VG**	**Plant: growth habit at tillering stage**					
QN	**B**	erect				EGA Bellaroi	1
		semi-erect				Jiloca, Kronos	3
		intermediate				Tamaroi, Valnova, Yallaroi	5
		semi-prostrate					7
		prostrate					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4. (*)	**GS 50-51 VG**	**Plant: time of ear emergence (first spikelet is visible on ears of 50% plants)**					
QN	**B**	very early					1
		early					3
		medium				Arrivato, Tamaroi, Yallaroi	5
		late				Kronos	7
		very late					9
5. (*)	**GS 55-59 VG**	**Flag leaf: glaucosity of sheath**					
QN	**B**	absent or very weak				Capeiti 8	1
		weak				Hyperno	3
		medium				Kalka	5
		strong				Arrivato, Yallaroi, Grandur, Jiloca	7
		very strong				Tamaroi, Valnova	9
6. (*)	**GS 55-59 VG**	**Flag leaf: glaucosity of lower side of leaf blade**					
QN	**B**	absent or very weak				EGA Bellaroi	1
		weak				Grandur, Hyperno	3
		medium				Esquilache	5
		strong				Bidi 17, Kalka	7
		very strong					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
7.	**GS 55-59 VG**	**Flag leaf: intensity of anthocyanin coloration of auricles**					
QN	**B**	absent or very weak				Kamilaroi, Tamaroi	1
		weak				Yallaroi	3
		medium					5
		strong				Wollaroi	7
		very strong					9

Comment: France has proposed to delete Characteristic 7. Fluctuating characteristic according to the year and the place.

8.	**GS 55-59 VS**	**Flag leaf: hairiness of auricle margin**					
QL	**B**	absent				Tamaroi	1
		present					9

Comment: France has proposed to delete Characteristic 8. Never done in France.

9.	**GS 58-60 VG**	**Awn: intensity of anthocyanin coloration**					
QN	**B**	absent or very weak				Hyperno, Kalka	1
		weak					3
		medium				Valnova	5
		strong					7
		very strong					9

Comment: France has proposed to delete Characteristic 9. Fluctuating characteristics according to the year and the place.

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
10. (+)	**GS 55-75 VS**	**Culm: hairiness of uppermost node**					
QN	**B**	absent or very weak				Andente, Bidi 17	1
		weak				Esquilache, Grandur, Tamaroi	3
		medium				Mexa, Yallaroi	5
		strong				Arrivato	7
		very strong					9
11. (*)	**GS 60-69 VG**	**Culm: glaucosity of neck**					
QN	**B**	absent or very weak				Capeiti 8	1
		weak					3
		medium				Andente	5
		strong				Roqueño, Tamaroi	7
		very strong				Kronos	9
12. (*)	**GS 60-69 VG**	**Ear: glaucosity**					
QN	**B**	absent or very weak				Capeiti 8	1
		weak				Jiloka, Kronos	3
		medium				Oscar ,Yallaroi	5
		strong				EGA Bellaroi, Grandur, Roqueño, Tamaroi	7
		very strong					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
13. (*)	**GS 75-92 MS**	**Plant: height (stem, ear and awn)**					
QN	**B**	very short				Gargiflash, Oscar	1
		short				Kamilaroi, Mexa	3
		medium				Grandur, Yallaroi	5
		tall				Capelli, Senatore, Tamaroi	7
		very tall					9
14. (*) (+)	**GS 70-92 VG**	**Ear: distribution of awns**					
QL	**B**	awnless					1
		tip awned				Saintly	2
		half awned					3
		fully awned				Arrivato, Tamaroi	4
Comment: France has proposed to delete Characteristic 14. No difference between varieties							
15. (*)	**GS 75-92 VG**	**Awns at tip of ear: length in relation to ear**					
QL	**B**	shorter				Saintly	1
		equal				Tamaroi	2
		longer				Arrivato, Oscar	3
16.	**GS 80-92 VS**	**Lower glume: shape (spikelet in mid-third of ear)**					
PQ	**B**	ovoid				Grandur, Kronos, Randur, Tamaroi	1
		elongated				Oscar, Yallaroi	2
		strongly elongated				Bidi-17, line4210.23.6	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
17. (+)	**GS 80-92 VS (as for 16)**	**Lower glume: shape of shoulder**					
PQ	**B**	sloping				Yallaroi	1
		rounded				Esquilache, Wollaroi	2
		straight				Hyperno, Roqueño	3
		elevated				Tamaroi	4
		elevated with a prominent 2nd beak				Capdur, Oscar, Saintly	5
18. (+)	**GS 80-92 VS (as for 16)**	**Lower glume: width of shoulder**					
QN	**B**	narrow				Oscar Tamaroi	3
		medium				Krono,s	5
		broad					7
19.	**GS 80-92 VS (as for 16)**	**Lower glume: length of beak**					
QN	**B**	very short				Jiloca, Saintly	1
		short				Tamaroi	3
		medium				Kailaroi	5
		long				Mexa	7
		very long					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
20. (+)	**GS 80-92 VS (as for 16)**	**Lower glume: shape of beak**					
PQ	**B**	straight				Durox, Mexa, Saintly	1
		slightly curved				Bidi 17, Hyperno, Tamaroi	3
		moderately curved				Capdur, Kamilaroi	5
		strongly curved					7
21. (*)	**GS 80-92 VS (as for 16)**	**Lower glume: hairiness of external surface**					
QL	**B**	absent				Grandur, Hyperno, Roqueño	1
		present				Paramo, Wollaroi	9
22. (*) (+)	**GS 90-92 VS**	**Straw: pith in cross section (half way between base of ear and stem node below)**					
PQ	**B**	thin				Hyperno, Valnova	3
		medium				Tamaroi	5
		thick				line4210.23.6, Paramo	7
23. (*)	**GS 90-92 VS**	**Awn: color**					
PQ	**B**	white				Esquilache, Kronos	1
		light brown				Kamailaroi, Yallaroi	2
		brown				line4210.23.6, Tejon	3
		black				Capdur, Tamaroi, Valnova	4

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24. (*)	**GS 90-92 MS**	**Ear: length (excluding awns)**					
QN	**B**	very short					1
		short				Arrivato, Kronos	3
		medium				Tamaroi	5
		long				Valnova	7
		very long					9
25. (*)	**GS 90-92 VS**	**Ear: color at maturity**					
PQ	**B**	white				Esquilache, Valdur, Yallaroi	1
		slightly colored				Randur	2
		strongly colored				Kronos, Tamaroi	3
26. (+)	**GS 92 VS**	**Ear: shape in profile view**					
PQ	**B**	tapering				Kalka	1
		parallel sided				Hyperno, Tamaroi	2
		semi-clavate					3
		clavate					4
		fusiform					5

Comment: France has proposed to delete Characteristic 26. No difference between varieties

27. (*) (+)	**GS 92 VS**	**Ear: density**					
QN	**B**	very lax					1
		lax				Kamilaroi	3
		medium				Kalka, Roqueño	5
		dense				Arrivato, Bidi-7	7
		very dense					9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
28. (*)	**GS 92 VS**	**Grain: color**					
PQ	**B**	white				Arrivato	1
		amber				Bellaroi, Hyperno	2
		light yellow				Tamaroi	3
Comment: France has proposed to delete Characteristic 28. Fluctuating characteristic according to the year and the place							
29. (*) (+)	**GS 92 VS**	**Grain: shape in dorsal view**					
PQ	**B**	round					1
		ovate				Arrivato	2
		oblong				Acme	3
		elliptical				Tamaroi	4
Comment: France has proposed to delete Characteristic 29. Never done in France							
30. (*) (+)	**GS 92 VS**	**Grain: length of brush hair in dorsal view**					
QN	**B**	short				Kalka, Chandur, Roqueño	3
		medium				Arrivato, Andente, Valdur	5
		long				Clairdoc	7
31. (+)	**GS 92 VS**	**Grain: shape in profile view**					
PQ	**B**	level					1
		sloping					2
		curved					3
Comment: France has proposed to delete Characteristic 31. Never done in France							

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
32.	**GS 92 MS**	**Grain: length**					
QN	**B**	short				Arrivato	3
		medium				Tamaroi	5
		long				EGA Bellaroi	7
Comment: France has proposed to delete Characteristic 32. Never done in France							
33.	**GS 92 MS**	**Grain: width**					
QN	**B**	narrow					3
		medium				Tamaroi	5
		wide				Yallaroi	7
Comment: France has proposed to delete Characteristic 33. Never done in France							
34. **(+)**	**GS 92 VS**	**Grain: germ shape**					
QL	**B**	oval				Tamaroi	1
		round				Arrivato	2
35. **(+)**	**GS 92 VS**	**Grain: germ face angle**					
QL	**B**	shallow				Tamaroi	1
		steep				Kalka	2
Comment: France has proposed to delete Characteristic 35. Never done in France							
36.	**GS 92 MG**	**Grain: weight (1000 grain wt.)**					
QN	**B**	low					3
		medium					5
		high					7
Comment: France has proposed to delete Characteristic 36. VCU characteristic.							

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
37. (+)	**GS 92 VS**	**Grain: coloration with phenol**					
QN	**C**	absent or very light				Esquilache, Hyperno	1
		light				Randur	3
		medium					5
		dark					7
		very dark					9
38. (*)	**GS 92 VG**	**Plant: seasonal type**					
PQ	**B**	winter type					1
		alternative type				Camacho, Valmora	2
		spring type				Kalka, Saintly, Tejon	3
39. (+)	**GS 92 MG**	**Glutenin composition: allele expression at locus Glu-A1**					
QL	**C**	band 1				EGA Bellaroi, Kamilaroi, Yallaroi	1
		band 2					2
		no band					3

Comment: France has proposed to delete Characteristic 39.

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
40. (+)	**GS 92 MG**	**Glutenin composition: allele expression at locus Glu-B1**					
QL	**C**	bands 6+8					1
		band 7					2
		bands 7+8				EGA Bellaroi, Wollaroi	3
		bands 7+16				Yallaroi	4
		bands 13+16					5
		bands 13+19					6
		bands 14+15					7
		bands 17+18					8
		band 20				Kamilaroi	9
		band 21					10
		band 22					11

Comment: France has proposed to delete Characteristic 40.

France has proposed to retain these two characteristics

	GS 7-51 VG	**Plant: frequency of plants with recurved flag leaves**		
QN		absent or very low	Roqueño	1
		low		3
		medium		5
		high		7
		very high	Capdur	9

Comment: This characteristic was deleted from this current draft as this is not a uniform characteristic and very much fluctuated by environment and the intermedicate states are difficult to define.

PQ	**GS 92 VG**	**Grain: shape**		
		ovoid		3
		semi-elongated	Tejon	5
		elongated	Capelli, Chandur, Senatore	5

Comment: This characteristic was replaced by Characteristic 29 in the current draft with better explanations and states

8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

8.2 Explanations for individual characteristics

(illustrations are provided where necessary):

Ad. 1: Coleoptile: intensity of anthocyanin coloration

Method for the Determination of Intensity of Anthocyanin Coloration

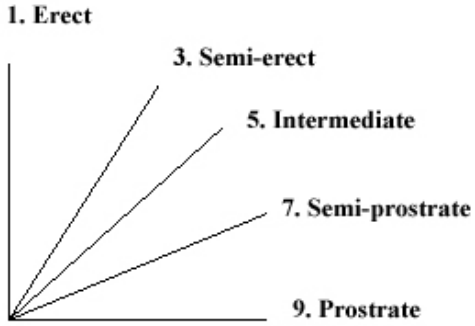
Number of grains per test	100 grains for distinctness and uniformity
Preparation of grains	Set up non-dormant grains on moistened filter paper with a Petri dish lid during germination.
Place	Laboratory or glasshouse.
Light	After the coleoptiles have reached a length of about 1 cm in darkness, they are placed in artificial light (daylight equivalent), 12,000 to 15,000 lux continuously for 3 - 4 days.
Temperature	15 to 20°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	At least one of the example varieties should be included as a control when testing for distinctness.

Ad. 2: First leaf: intensity of anthocyanin coloration

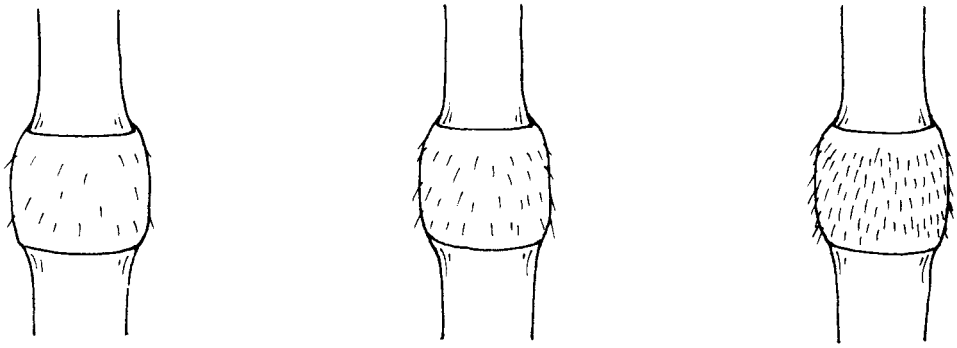
The plants should be grown in the glasshouse on neutral substrate (for example sand) at a temperature of 18°C and at 15000 Lux continuous illumination from the time of appearance of the coleoptile. The color of the substrate should be preferably pale to get a better contrast for the observation. The intensity of anthocyanin coloration should be observed at exactly stage 10 as the expression may disappear thereafter.

Ad. 3: Plant: growth habit at tillering stage

The growth habit at tillering stage (growth stages 25-29) 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 middle axis should be used.

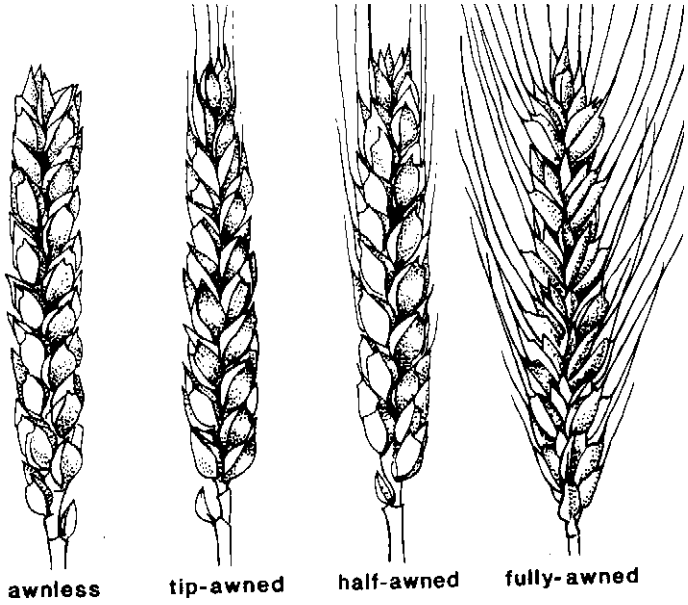


Ad. 10: Culm: hairiness of uppermost node



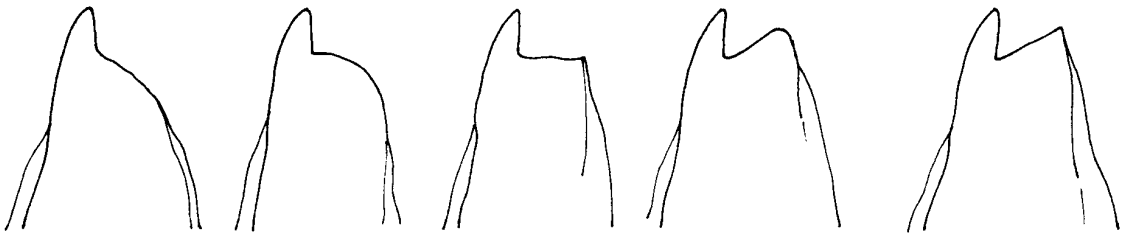
3	5	7
weak	medium	strong

Ad. 14: Ear: distribution of awns



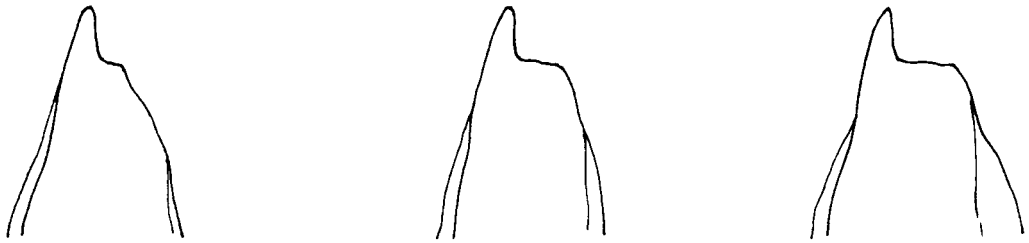
1 2 3 4

Ad. 17: Lower glume: shape of shoulder



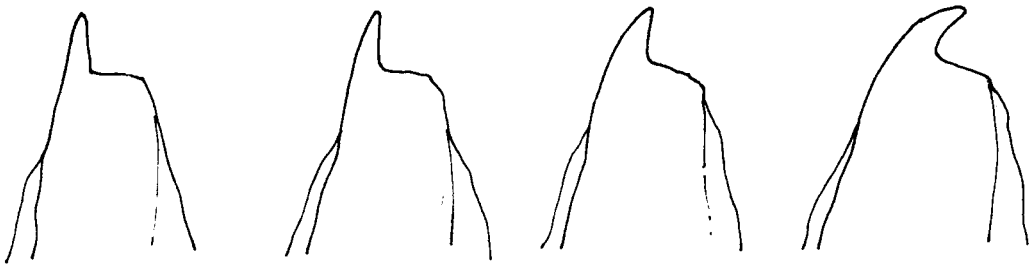
1	2	3	4	5
sloping	rounded	straight	elevated	elevated with 2nd beak present

Ad. 18: Lower glume: width of shoulder



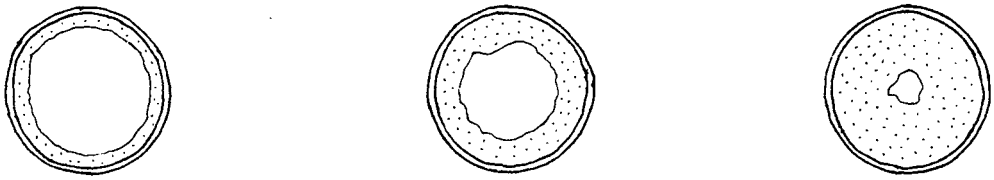
3	5	7
narrow	medium	broad

Ad. 20: Lower glume: shape of beak



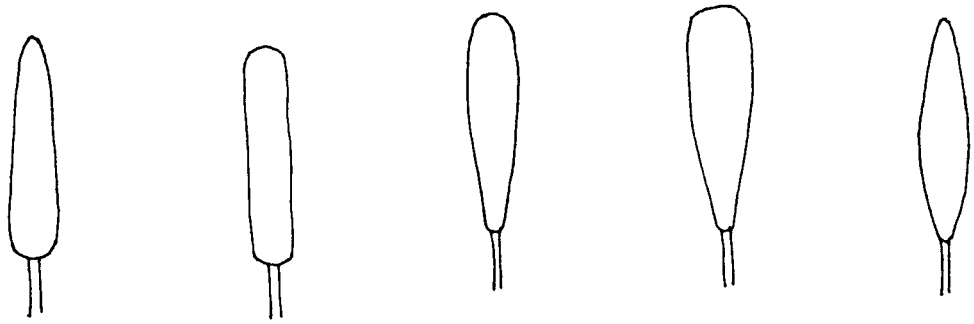
1 straight | 2 slightly curved | 3 moderately curved | 4 strongly curved

Ad. 22: Straw: pith in cross section (half way between base of ear and stem node below)



3 thin | 5 medium | 7 thick

Ad. 26: Ear: shape in profile view



1 tapering | 2 parallel sided | 3 semi-clavate | 4 clavate | 5 fusiform

Ad. 27: Ear: density

1	3	5	7	9
very lax	lax	medium	dense	very dense

(Comments from Portugal)
Ear: density (distance between the base of the 3rd and the 8th spikelet)

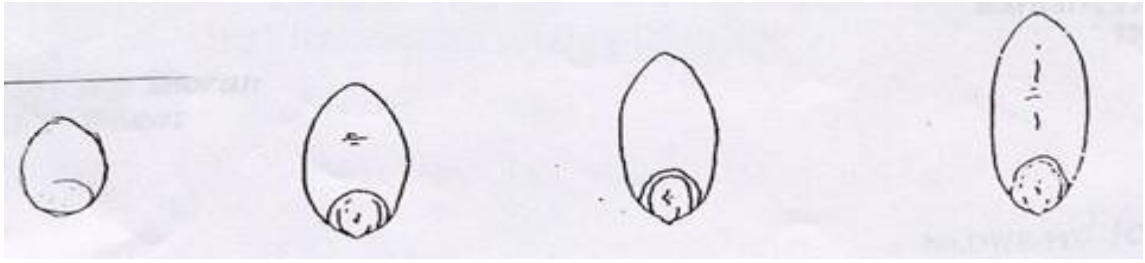
very lax	*1*	*>27 mm*
lax	*3*	*23-27 mm*
medium	*5*	*18-22 mm*
dense	*7*	*13-17 mm*
very dense	*9*	*< 13 mm*

Ad. 28: Grain: color
Ad. 29: Grain: shape in dorsal view
Ad. 30: Grain: length of brush hair in dorsal view
Ad. 31: Grain: shape in profile view
Ad. 32: Grain: length
Ad. 33: Grain: width
Ad. 34: Grain: germ shape
Ad. 35: Grain: germ face angle

All grain characteristics need to be observed on well filled grains that have not come from plants stressed during grain filling. For assessing following grain characteristics a hand lens with 10x magnification is recommended.

Ad. 29: Grain: shape in dorsal view:

When grain is viewed from above the shape can be described in following four ways:

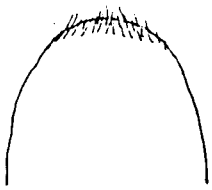


1	2	3	4
round	ovate	oblong	elliptical

Ad. 30: Grain: length of brush hair in dorsal view

Brush hair length is viewed from the top of the grain and can be described in the following ways:

3
short

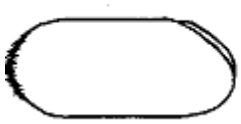


5
medium

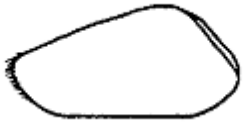
7
long

Ad. 31: Grain: shape in profile view

When the grain is viewed from the side the back shape can be described in following three ways:



1
level



2
sloping

3
curved

Ad. 34: Grain: germ shape

The germ (or embryo) is at the bottom end of the grain for the purpose of variety descriptions the germ includes the whole area of humps and bulges. The grain should be viewed obliquely and is classified by the overall shape of the germ area in following two ways:

1
oval

2
round

Ad. 35: Grain: germ face angle

The germ (or embryo) face angle is viewed from the side and is described as being steep if the angle (when the grain sits flat on its base) is greater than 45°. A shallow germ face angle is less than 45° from the horizontal:

1
shallow

2
steep

Ad. 37: Grain: coloration with phenol

Method for Determination of Phenol Reaction

Number of grains per test	100 grains for distinctness and uniformity. 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	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 37 in the Table of Characteristics.
Note	At least one of the example varieties should be included as a control.

Ad. 39: Glutenin composition: allele expression at locus Glu-A1
Ad. 40: Glutenin composition: allele expression at locus Glu-B1

For the analysis of high molecular weight glutenin subunit (HMW-GS), polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS PAGE) should be used. Glutenins are encoded in tetraploid wheat by two compound loci, known as Glu-A1, Glu-B1 on the long arms of the group 1 chromosomes. There are a number of alleles at each locus and the analysis of HMW glutenins is based on the recognition of these alleles from proteins, which appear on gels as a series of well defined bands or patterns of bands. The alleles are described by band numbers according to the definition given to them by Payne and Lawrence, (1983).

Description of the Method to be Used

Glutenin composition: allele expression at loci Glu-A1 (27), Glu-B1 (28)

SDS PAGE Method for Analysis of HMW Glutenins from *Triticum turgidum* ssp. *durum*

Apparatus and equipment

Any suitable vertical electrophoresis system can be used, provided that the gels can be kept at a constant temperature. A gel thickness of no more than 1.5 mm is recommended. The power supply used should be capable of delivering both constant current and constant voltage output.

Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

Acrylamide (specially purified for electrophoresis)
Bisacrylamide (specially purified for electrophoresis)
Tris (hydroxymethyl) methylamine (TRIS)
Sodium dodecyl sulphate (SDS)
Ammonium persulphate (APS)
2-mercaptoethanol
TEMED (NNN'N'-tetramethylethylenediamine)
Trichloroacetic acid (TCA)
Hydrochloric acid
Glacial acetic acid
Glycine
n-Butanol
Pyronin Y (or G)
Glycerol (d = 1.256)
Methanol or ethanol
Coomassie Brilliant Blue R-250 (or equivalent)
Coomassie Brilliant Blue G-250 (or equivalent)

Solutions

Extraction solution

Extraction of glutenins only

Stock solution:

6.25 ml 1M TRIS HCl buffer, PH 6.8 (see 3.3.2)
12.05 ml distilled water
2g SDS
10 mg Pyronin Y (or G)
10 ml glycerol
This solution can be stored for two months at 4°C.

Immediately before use, extraction solution is prepared as follows:

4.25 ml stock solution (above) plus 0.75 ml 2-mercaptoethanol made up to 10.0 ml with distilled water. This solution must be prepared immediately prior to use and cannot be stored.

Extraction of glutenins following gliadins

Solution A - 25 ml 2 - chloroethanol + 50 mg Pyronin Y/G, made up to 100 ml with distilled water.

Solution B - 27.0 g urea, 3.0 ml 2 - mercaptoethanol + 10.0 g SDS, made up to 100 ml with distilled water.

Electrophoresis (running) buffer

Stock solution:

141.1 g glycine
30.0 g TRIS
10.0 g SDS
made up to 1 1 with distilled water.
Immediately before use, the stock solution is diluted 1:10 with distilled water.

The stock buffer solution can be stored for 2 months at room temperature. Do not store the diluted buffer more than one week. The pH of the buffer must be close to 8.3.

Gel preparation solutions

Stock resolving gel buffer (1M TRIS HCl, pH 8.8)

121.14 g TRIS plus approximately 20 ml HCl (d = 1.19) made up to 1 litre with distilled water. This buffer can be stored at 4°C for 2 months.

Stock stacking gel buffer (1M TRIS HCl, pH 6.8)

121.14 g TRIS plus approximately 78 ml HC1 (d = 1.19) made up to 1 litre with distilled water. This buffer can be stored at 4°C for 2 months.

10% (w/v) SDS solution

10g of SDS dissolved in distilled water and made up to 100 ml. This solution can be stored at 4°C for 2 months. Prior to use, stir and heat gently to re-dissolve the SDS, if it comes out of solution.

1% (w/v) ammonium persulphate solution

1g of APS dissolved in distilled water and made up to 100 ml. This solution must be prepared immediately prior to use.

Stock acrylamide solution

40.02g acrylamide made up to 100 ml with distilled water.

Stock bisacrylamide solution

0.5198g bisacrylamide made up to 130 ml with distilled water.

Staining solutions

0.25g Coomassie Brilliant Blue G-250 plus 0.75g Coomassie Brilliant Blue R-250, made up to 100 ml with water.

55g TCA, 65 ml glacial acetic acid, 180 ml methanol or ethanol plus 25 ml solution, made up to 1litre with distilled water.

Procedure

Protein extraction

Glutenins only

Individual seeds are ground using a hammer (or other device). Ground seed meal is mixed with diluted sample extraction buffer (3.1.1) in a 3 ml polypropylene hemolyse or similar tube with a screw-on or fitted cap. The ratio of meal/extraction buffer is 50 mg/0.75 ml. The samples are extracted for 2 hours at room temperature, mixed several times using a vortex mixer, heated in a boiling water bath for 10 minutes and then allowed to cool. The tubes are centrifuged at 18000g for 5 minutes.

Glutenins following gliadins

If desired, glutenins and gliadins can be analyzed from the same grain. Gliadins are extracted first by adding 0.25 ml of Solution A (3.1.2) to a crushed grain (or half-grain) in a microtiter plate or micro-centrifuge tube and incubating overnight at room temperature. Following this, glutenins are extracted by adding 0.5 ml of Solution B (3.1.2) to the crushed grain and incubating overnight at room temperature.

According to the gel thickness and the size of the wells, the volume of extract loaded can vary. Between 10 and 25 µl is usually sufficient.

Preparation of the gel

Clean and dry gel cassettes are assembled, according to the design of the equipment used. If tape is used to seal the cassettes, it is advisable to assemble them at least one day in advance of use, to enable the tape to 'age' and adhere better.

Resolving (main) gel (10% acrylamide, pH 8.8)

To make two slab gels of 180 x 160 x 1.5 mm, the following is required:

20 ml stock acrylamide solution (3.3.5)
26 ml stock bisacrylamide solution (3.3.6),
30 ml stock gel buffer (3.3.1).

These should be at room temperature. The mixture is degassed in a 100 ml Büchner flask for 2 - 3 minutes. To this is added:

2 ml APS (3.3.4),
0.8 ml SDS (3.3.3),
40 µl TEMED (use straight from bottle).

The gels are then carefully poured, avoiding the formation of air bubbles, and polymerization allowed to take place at room temperature.

The gel cassettes should not be filled entirely, in order to leave room for a 3-4 cm layer of stacking gel. The gel surface is carefully overlaid with n-butanol (or distilled water) using a syringe. When polymerization is finished (about 30 min.), the gel surface is carefully rinsed with distilled water and dried with filter paper.

Resolving (main) gel (7% acrylamide, pH 8.8)

To resolve the sub-units 2 and 2*, it is necessary to use main gels of 7% acrylamide concentration.

To make two slab gels of 180 x 160 x 1.5 mm, the following is required:

14 ml stock acrylamide solution (3.3.5)
6 ml distilled water
26 ml stock bisacrylamide solution (3.3.6),
30 ml stock gel buffer (3.3.1).

These should be at room temperature. The mixture is de-gassed in a 100 ml Büchner flask for 2 - 3 minutes. To this is added:

2 ml APS (3.3.4),
0.8 ml SDS (3.3.3),
40 µ TEMED (use straight from bottle).

The gels are then carefully poured, avoiding the formation of air bubbles, and polymerization allowed to take place at room temperature.

The gel cassettes should not be filled entirely, in order to leave room for a 3-4 cm layer of stacking gel. The gel surface is carefully overlaid with n/butanol (or distilled water) using a syringe. When polymerization is finished (about 30 min.), the gel surface is carefully rinsed with distilled water and dried with filter paper.

Stacking gel (3% acrylamide, pH 6.8)

In a 50 ml Büchner flask, mix:

1.50 ml stock acrylamide solution (3.3.5),
2.15 ml stock bisacrylamide solution (3.3.6)
2.50 ml stock gel buffer (3.3.2) and
13.15 ml distilled water.

Following de-gassing add:

0.75 ml APS (3.3.4),
0.2 ml SDS (3.3.3),
15 µl TEMED (straight from bottle)

Mix carefully and immediately pour the stacking gels to the top of the gel cassettes. Insert the well-forming "comb", avoiding air bubbles. Allow to polymerize for about 2 hours at room temperature. The "combs" are then removed carefully from the gel cassettes and the wells rinsed using diluted electrophoresis running buffer (3.2).

Electrophoresis

The tank is filled with the appropriate volume of running buffer (3.2), cooled to 15°C. Following sample loading, electrophoresis is carried out at a constant current of 8 mA/cm^2 (cross-sectional area) of gel until the pyronin Y/G has moved through the stacking gel, and then at 16 mA/cm^2 of gel (maximum voltage 300V) until the marker is at the bottom of the gel. The temperature should be maintained at 15°C.

Fixing and staining

The gel cassettes are removed from the tank, opened and the gels fixed in 250 ml of 15% (w/v) TCA for at least 30 minutes. The gels are rinsed in distilled water and stained overnight in 250 ml of staining solution (3.4.2) at room temperature. Destaining is not usually necessary but gels should be washed in distilled water before being stored in sealed polythene bags.

Other staining procedures can be successfully used (e.g. Coomassie Brilliant Blue G or equivalent in TCA alone). The final quality control criterion, both for gel preparation and gel staining, is to analyze the suggested example varieties on each batch of gels. The separation of the suggested bands, and their relative electrophoretic mobilities (molecular weights) must be clear in order for the procedures to be judged satisfactory.

The descriptions of the growth stages of the Zadoks decimal code for cereals.

0	**Germination**	**5**	**Inflorescence (ear/panicle) emergence**
00	Dry seed	50	--
01	Start of imbibition (water absorption)	51	First spikelet of inflorescence just visible
02	--	52	--
03	Imbibition complete	53	1/4 of inflorescence emerged
04	--	54	--
05	Radicle (root) emerged from caryopsis (seed)	55	1/2 of inflorescence emerged
06	--	56	--
07	Coleoptile	57	3/4 of inflorescence emerged
08	--	58	--
09	Leaf just at coleoptile tip	59	Emergence of inflorescence
1	**Seedling growth**	**6**	**Anthesis (flowering)**
10	First leaf through coleoptile	60	--
11	First leaf unfolded	61	Beginning of anthesis
12	2 leaves unfolded	62	--
13	3 leaves unfolded	63	--
14	4 leaves unfolded	64	--
15	5 leaves unfolded	65	Anthesis half-way
16	6 leaves unfolded	66	--
17	7 leaves unfolded	67	--
18	8 leaves unfolded	68	--
19	9 or more leaves unfolded	69	Anthesis complete
2	**Tillering**	**7**	**Milk development**
20	Main shoot only	70	--
21	Main shoot and 1 tiller	71	Caryopsis (kernel) water ripe
22	Main shoot and 2 tillers	72	--
23	Main shoot and 3 tillers	73	Early milk
24	Main shoot and 4 tillers	74	--
25	Main shoot and 5 tillers	75	Medium milk

26	Main shoot and 6 tillers	76	--
27	Main shoot and 7 tillers	77	Late milk
28	Main shoot and 8 tillers	78	--
29	Main shoot and 9 or more tillers	79	--
3	**Stem elongation**	**8**	**Dough development**
30	Pseudostem (leaf sheath) erection	80	--
31	First node detectable	81	--
32	2nd node detectable	82	--
33	3rd node detectable	83	Early dough
34	4th node detectable	84	--
35	5th node detectable	85	Soft dough
36	6th node detectable	86	
37	Flag leaf just visible	87	Hard dough
38	--	88	--
39	Flag leaf ligule just visible	89	--
4	**Booting**	**9**	**Ripening**
40	--	90	--
41	Flag leaf sheath extending	91	Caryopsis hard (difficult to divide)
42	--	92	Caryopsis hard (not dented by thumbnail)
43	Boots just visibly swollen	93	Caryopsis loosening in daytime
44	--	94	Over-ripe, straw dead and collapsing
45	Boots swollen	95	Seed dormant
46	--	96	Viable seed giving 50% germination
47	Flag leaf sheath opening	97	Seed not dormant
48	--	98	Secondary dormancy induced
49	First awns visible	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 Evaluation. Kluwer Academic Publishers, NL, 41: 47-54.

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

Naghavi, M.R., Monfared, R.S., Ahkami, A.H., Ombidbakhsh, M.A., 2009. Genetic Variation of Durum Wheat Landrace and Cultivars Using Morphological and Protein Markers, Proceedings of World Academy of Science, Engineering and Technology, Volume 37, January 2009 (ISSN-3740), Dubai, AE.

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

10. Technical Questionnaire

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

	Application date: (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire

1.1 Botanical name	*Triticum turgidum* subsp. *durum* (Desf.) Husn.
1.2 Common name	Durum Wheat

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	
Breeder (if different from applicant)	

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

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

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

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)

(b) partially known cross [ ]
(please state known parent variety(ies))

(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

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

4.1.4 Other [ ]
(please provide details)

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

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

4.2 Method of propagating the variety

In the case of hybrid varieties the production scheme for the hybrid should be provided on a separate sheet. This should provide details of all the parent lines required for propagating the hybrid e.g.

Single Hybrid

(… female parent …) x (… male parent …)

Three-Way Hybrid

(… female line …) x (… male line …)

=> single hybrid used as female parent x (… male parent …)

and should identify in particular:

(a) any male sterile lines
(b) maintenance system of male sterile lines.

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

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

	Characteristics	Example Varieties	Note
5.1 (4)	**Plant: time of ear emergence (first spikelet is visible on ears of 50% plants)**		
	very early		1[ ]
	early		3[ ]
	medium	Arrivato, Tamaroi, Yallaroi	5[ ]
	late	Kronos	7[ ]
	very late		9[ ]
5.2 (13)	**Plant: height (stem, ear and awn)**		
	very short	Gargiflash, Oscar	1[ ]
	short	Kamilaroi, Mexa	3[ ]
	medium	Grandur, Yallaroi	5[ ]
	tall	Capelli, Senatore, Tamaroi	7[ ]
	very tall		9[ ]
5.3 (14)	**Ear: distribution of awns**		
	awnless		1[ ]
	tip awned	Saintly	2[ ]
	half awned		3[ ]
	fully awned	Arrivato, Tamaroi	4[ ]
5.4 (21)	**Lower glume: hairiness of external surface**		
	absent	Grandur, Hyperno, Roqueño	1[ ]
	present	Paramo, Wollaroi	9[ ]

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

	Characteristics	Example Varieties	Note
5.5 (22)	**Straw: pith in cross section (half way between base of ear and stem node below)**		
	thin	Hyperno, Valnova	3[ ]
	medium	Tamaroi	5[ ]
	thick	line4210.23.6, Paramo	7[ ]
5.6 (25)	**Ear: color at maturity**		
	white	Esquilache, Valdur, Yallaroi	1[ ]
	slightly colored	Randur	2[ ]
	strongly colored	Kronos, Tamaroi	3[ ]
5.7 (38)	**Plant: seasonal type**		
	winter type		1[ ]
	alternative type	Camacho, Valmora	2[ ]
	spring type	Kalka, Saintly, Tejon	3[ ]

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

6. Similar varieties and differences from these varieties

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

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example	*Ear: color at maturity*	*white*	*strongly colored*

Comments:

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

[#]7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [ ] No [ ]

(If yes, please provide details)

7.3 Other information

A representative color photograph of the variety should accompany the Technical Questionnaire.

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

If the answer to (b) is yes, please attach a copy of the authorization.

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

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

9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a) Microorganisms (e.g. virus, bacteria, phytoplasma) Yes [ ] No [ ]

(b) Chemical treatment (e.g. growth retardant, pesticide) Yes [ ] No [ ]

(c) Tissue culture Yes [ ] No [ ]

(d) Other factors Yes [ ] No [ ]

Please provide details for where you have indicated “yes”.

……

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant’s name

Signature Date

[End of document]