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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

WHEAT

UPOV Code(s): TRITI_AES

Triticum aestivum L. emend. Fiori et Paol.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from France

to be considered by the

Technical Working Party for Agricultural Crops
at its forty-fifth session, to be held in Mexico City, Mexico,
from 2016-07-11 to 2016-07-15

Disclaimer: this document does not represent UPOV policies or guidance

Alternative names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Triticum aestivum L. emend. Fiori et Paol.	Wheat	Blé	Weizen	Trigo

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.

* These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Triticum aestivum* L. emend. Fiori et Paol.

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 seeds and ears (if requested).

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

Seeds: 3 kg
Ears (if requested): 120

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.

The ear should be well developed and should contain a sufficient number of viable seeds to establish a satisfactory row of plants for observation.

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 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 in Chapter 8.

3.4 Test Design

3.4.1 Each test should be designed to result in a total of at least 2000 plants, which should be divided between at least 2 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.

3.4.3 To read: 3.4.2 If tests on ear rows are conducted, at least 100 ear rows should be observed.

3.4.3 The assessment of the characteristic "Seasonal type" should be carried out on at least 300 plants.

3.4.4 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

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.

To assess distinctness of hybrids, the parent lines and the formula may be used according to the following recommendations:

(i) description of parent lines according to the Test Guidelines;

(ii) check of the originality of the parent lines in comparison with the variety collection, based on the characteristics in Chapter 7, in order to identify similar parent lines;

(iii) check of the originality of the hybrid formula in relation to the hybrids in the variety collection, taking into account the most similar lines; and

(iv) assessment of the distinctness at the hybrid level for 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 Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.1.4 Number of plants or parts of plants to be Examined

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 taken from single plants, the number of parts to be taken from each of the plants should be 1.

4.1.5 Method of Observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the second 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. color 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

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 The assessment of uniformity for hybrid varieties depends on the type of hybrid and should be according to the recommendations for hybrid varieties in the General Introduction.

4.2.3 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.

4.2.4 For the assessment of uniformity of 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. A an ear-row is considered to be an off-type ear-row if there is more than one off-type plant within that ear-row.

4.2.5 Paragraph 4.2.4. to 4.2.8 should read as follows:

4.2.4 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 plants/parts of plants

B sample size of 2000 plants or parts of plants

4.2.5 For the assessment of uniformity in a sample of 2000 plants, a population standard of 0.3% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 2000 plants, 10 off-types are allowed.

4.2.6 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.

4.2.7 For "A" characteristics, with the exception of characteristic 2 and 3, the assessment of uniformity can be done in 2 steps. In a first step, 20 plants are observed. If no off-types are observed, the variety is considered to be uniform. If more than 3 off-types are observed, the variety is considered 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.

4.2.8 For the assessment of uniformity of hybrid varieties, a population standard of 10% and an acceptance probability of at least 95% should be applied. In case of characteristics indicated by B, the sample size for the assessment of uniformity may be reduced to 200 plants. In case of a sample size of 200 plants, 27 off-types are allowed. In case of a sample size of 100 ear-rows, plants or parts of plants, 15 off-types are allowed.

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 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 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) Lower glume: hairiness on external surface (characteristic 12)
(b) Ear: scurs or awns (characteristic 17)
(c) Ear: color (characteristic 19)
(d) Seasonal type (characteristic 27)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 “Examining Distinctness”.

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

6.2.1 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.

6.2.2 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), 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

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

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.

(w): winter type variety
(s): spring type variety

6.5 Legend

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1 2	**3 4**	**5 6**	**7**			
	Name of characteristics in English	**Nom du caractère en français**	**Name des Merkmals auf Deutsch**	**Nombre del carácter en español**		
	states of expression	types d'expression	Ausprägungsstufen	tipos de expresión		

1 Characteristic number

2 (*) Asterisked characteristic – see Chapter 6.1.2

3 Type of expression
QL Qualitative characteristic – see Chapter 6.3
QN Quantitative characteristic – see Chapter 6.3
PQ Pseudo-qualitative characteristic – see Chapter 6.3

4 Method of observation (and type of plot, if applicable)
MG, MS, VG, VS – see Chapter 4.1.5

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

6 (a)-(a) See Explanations on the Table of Characteristics in Chapter 8.1

7 Growth stage key See Explanations on the Table of Characteristics in Chapter 8

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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.	**PQ VG|A**	**(+)**	**00**				
		Seed: color					
		white				Blini (s), SY Ideo (w)	1
		reddish				Granary (s), Solehio (w)	2
		purple				Indigo (w)	3
		bluish				Skorpion (w)	4
2.	**QN VG|A**	**(+)**	**00**				
		Seed: coloration with phenol					
		absent or very light	nulle ou très faible	fehlend oder sehr hell	ausente o muy ligera	Bitop (w)	1
		light	faible	hell	ligera	Lavett (s), SY Ideo (w)	3
		medium	moyenne	mittel	media	Sensas (s), SY Moisson (w)	5
		dark	forte	dunkel	oscura	Antonius (w), Granary (s)	7
		very dark				Callobre (w), Lennox (s)	9
3.	**QN VG|A**	**(+)**	**09-11**				
		Coleoptile: anthocyanin coloration					
		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Cornetto (s), Rubisko (w)	1
		weak	faible	gering	débil	Antonius (w), FD 1 24 (s)	3
		medium	moyenne	mittel	media	Maxwell (w), Specifik (s)	5
		strong	forte	stark	fuerte	Sensas (s), SY Ideo (w)	7
		very strong	très forte	sehr stark	muy fuerte	Cellule (w)	9
4.	**(*) QN VG|B**	**(+)**	**25-29**				
		Plant: growth habit					
		erect					1
		semi erect				Callobre (w), CH Campala (s)	3
		intermediate				Apache (w), Sensas (s)	5
		semi prostrate				Olivart (s), Solehio (w)	7
		prostrate				Stelarka (w)	9

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5.		**QN**	**VG|B**	**(+)**		**47-51**			
		Plant: frequency of plants with recurved flag leaves							
		absent or very low						Genius (w)	1
		low						Solehio (w), Triso (s)	3
		medium						Callobre (w), Specifik (s)	5
		high						Antonius (w), Blini (s)	7
		very high						Atacama (w), FD 1 24 (s)	9
6.		**QN**	**VG|B**	**(+)**		**49-60**			
		Flag leaf: anthocyanin coloration of auricles							
		absent or weak						Soissons (w), Triso (s)	1
		medium						Antille (s), Raffy (w)	2
		strong						Astardo (w), Dollar (s)	3
7.	**(*)**	**QN**	**MG|B**	**(+)**		**50**			
		Time of ear emergence							
		very early						Badiel (s), Maxwell (w)	1
		early						Sensas (s), Solehio (w)	3
		medium						Granary (s), Sertori (w)	5
		late						Rosario (w), Triso (s)	7
		very late						Adequat (w)	9
8.	**(*)**	**QN**	**VG|B**			**60-65**			
		Flag leaf: glaucosity of sheath							
		absent or very weak						Basilio (w)	1
		weak						CH Campala (s), Saturnus (w)	3
		medium						Bastian (s), Maxwell (w)	5
		strong						Solehio (w), Triso (s)	7
		very strong						Waximum (w)	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
9.		**QN VG|B**	**(+)**	**60-65**			
		Flag leaf: glaucosity of blade					
		absent or very weak				Courtot (w)	1
		weak				FD 1 24 (s), Saturnus (w)	3
		medium				Blini (s), SY Moisson (w)	5
		strong				Lennox (s), SY Ideo (w)	7
		very strong				Waximum (w)	9
10.	**(*)**	**QN VG|B**		**60-69**			
		Ear: glaucosity					
		absent or very weak				Soissons (w)	1
		weak				Callobre (w), Panifor (s)	3
		medium				Granary (s), Solehio (w)	5
		strong				Edgar (w), Specifik (s)	7
		very strong				Waximum (w)	9
11.		**QN VG|B**		**60-69**			
		Culm: glaucosity of neck					
		absent or very weak				Basilio (w)	1
		weak				CH Campala (s), Soissons (w)	3
		medium				Granary (s), Ronsard (w)	5
		strong				Lennox (s), SY Moisson (w)	7
		very strong				Waximum (w)	9
12.	**(*)**	**QL VG|B**	**(a)**	**69-92**			
		Lower glume: hairiness on external surface					
		absent				Soissons (w), Triso (s)	1
		present				Franz (w), Galera (s)	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
13.	**(*)**	**QN MG|B**	**(+)**	**75-92**			
		Plant: length					
		very short				Fronton (w)	1
		short				Apache (w), Lennox (s)	3
		medium				FD 1 24 (s), Solehio (w)	5
		long				Antonius (w)	7
		very long				Capo (w)	9
14.	**(*)**	**QN VG|A**	**(+)**	**80-92**			
		Straw: pith in cross section					
		thin				FD 1 24 (s), SY Moisson (w)	1
		medium				Apache (w), KWS Flint (s)	2
		thick or filled				Olivart (s), Synchro (w)	3
15.	**(*)**	**QN MS|B/VG|B**	**(+)**	**80-92**			
		Ear: density					
		very lax					1
		lax				Kranich (w), Lennox (s)	3
		medium	demi-lâche à demi-compact	mittel	media	Granary (s), Solehio (w)	5
		dense	compact	dicht	densa	Cellule (w), Virgile (s)	7
		very dense					9
16.		**QN MS|B/VG|B**	**(+)**	**80-92**			
		Ear: length					
		very short				Olivart (s)	1
		short				GK Berény (w), Granary (s)	3
		medium				Rubisko (w), Sensas (s)	5
		long				Specifik (s), SY Ideo (w)	7
		very long				Edgar (w)	9
17.	**(*)**	**QL VG|B**	**(+)**	**80-92**			
		Ear: scurs or awns					
		both absent				Gorda (s)	1
		scurs present				Apache (w), Granary (s)	2
		awns present				Sensas (s), Solehio (w)	3

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18.	**(*)**	**QN MS|B/VG|B**	**(+)**	**80-92**			
		Ear: length of scurs or awns					
		very short				Homeros (w), KWS Flint (s)	1
		short				Apache (w), Tybalt (s)	3
		medium				SY Ideo (w)	5
		long				Courtot (w), Granary (s)	7
		very long				FD 1 24 (s), SY Moisson (w)	9
19.	**(*)**	**QL VG|B**	**(+)**	**80-92**			
		Ear: color					
		white				Granary (s), Solehio (w)	1
		colored				Bastian (s), Sertori (w)	2
20.		**PQ VG|B**	**(+)**	**80-92**			
		Ear: shape in profile					
		tapering				Solveig (w), Tybalt (s)	1
		parallel sided				Granary (s), Solehio (w)	2
		slightly clavate				Homeros (w)	3
		strongly clavate				Vulcanus (w)	4
		fusiform				Apache (w), FD 1 24 (s)	5
21.		**QN VG|A**	**(+)**	**(a)** **80-92**			
		Apical rachis segment: area of hairiness on convex surface					
		absent or very small				Soissons (w)	1
		small				Solehio (w), Specifik (s)	3
		medium				Granary (s), Homeros (w)	5
		large				Kranich (w), KWS Bittern (s)	7
		very large				Mv Bodri (w)	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
22.		**QN VG|A**	**(+) (a)**	**80-92**			
		Lower glume: shoulder width					
		absent or very narrow				Courtot (w)	1
		narrow				Soissons (w), Tybalt (s)	3
		medium				Sensas (s), Solehio (w)	5
		broad				KWS Collada (s), Sosthene (w)	7
		very broad					9
23.		**QN VG|A**	**(+) (a)**	**80-92**			
		Lower glume: shoulder shape					
		strongly sloping				Amulett (s), Courtot (w)	1
		slightly sloping				Solehio (w), Tybalt (s)	3
		horizontal				Lennox (s), Solveig (w)	5
		slightly elevated				Sosthene (w), Virgile (s)	7
		strongly elevated					9
24.		**QN MS|A/VG|A**	**(+) (a)**	**80-92**			
		Lower glume: length of beak					
		very short				Solveig (w)	1
		short				Kranich (w), Tybalt (s)	3
		medium				Blini (s), Sotchy CS (w)	5
		long				Sensas (s), Soissons (w)	7
		very long				FD 1 24 (s), Rubisko (w)	9
25.	**(*)**	**QN VG|A**	**(+) (a)**	**80-92**			
		Lower glume: shape of beak					
		straight				FD 1 24 (s), Solveig (w)	1
		slightly curved				Cellule (w), Granary (s)	3
		moderately curved				Edgar (w)	5
		strongly curved				Sertori (w)	7
		geniculate				Velocity (w)	9

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
26.		**QN**	**VG|A**	**(+)**	**(a)**	**80-92**			
		Lower glume: area of hairiness on internal surface							
		very small						Lupus (w)	1
		medium						KWS Scirocco (s), Solehio (w)	3
		very large						Apache (w), Lennox (s)	5
27.	**(*)**	**PQ**	**VG**	**(+)**					
		Seasonal type							
		winter type						Solehio (w)	1
		alternative type						SY Moisson (w)	2
		spring type						Lennox (s)	3

8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

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

(a) Characteristics of lower glume should be observed on spikelets in the midthird of ear.

8.2 Explanations for individual characteristics

Ad. 1: Seed: color

The seed color should be observed on dry seeds or by using NaOH solution (seeds soaked for 10 minutes at 60°C or 60 minutes at room temperature in a 5M NaOH solution).

Ad. 2: Seed: coloration with phenol

The seed coloration with phenol cannot be observed on purple nor bluish seeds.

Method for Determination of Phenol Reaction:
Number of seeds per test: 100 seeds. The seeds should not have been treated chemically.
Preparation of seeds: Soak in tap water for 16 to 20 hours, drain and remove surface water, place the seeds with crease downwards, cover dish with lid
Concentration of solution: 1 per cent Phenol-solution (freshly made up)
Amount of solution: The seeds 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)
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. 3: Coleoptile: anthocyanin coloration

Method for the Determination of Anthocyanin Coloration
Number of seeds per test: 100 seeds
Preparation of seeds: Set up non-dormant seeds on moistened filter paper covered with a Petri dish lid during germination
Place: Laboratory or greenhouse
Light: After the coleoptiles have reached a length of about 1 cm in the dark, they are placed in artificial light (daylight equivalent) at 13000 to 15000 lux continuously for 3-4 days
Temperature: 15 to 20°C
Time of recording: Coleoptiles fully developed (about 1 week) at 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. 4: Plant: growth habit

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.

1	3	5	7	9
erect	semi-erect	intermediate	semi-prostrate	prostrate

Ad. 5: Plant: frequency of plants with recurved flag leaves

1 (absent or very low): all or almost all flag leaves are rectilinear
3 (low): about 1/4 of the plants with recurved flag leaves
5 (medium): about 1/2 of the plants with recurved flag leaves
7 (high): about 3/4 of the plants with recurved flag leaves
9 (very high): almost all or all flag leaves are recurved

Ad. 6: Flag leaf: anthocyanin coloration of auricles

The appropriate scoring time between stages 49 and 60 should be determined depending on the location. All varieties should be assessed at the same stage.

Ad. 7: Time of ear emergence

Time of ear emergence is reached when the first spikelet is visible on 50% of ears.

Ad. 9: Flag leaf: glaucosity of blade

Observations should be made on the lower side of the blade.

Ad. 13: Plant: length

The length of plant includes stem, ear, awns and scurs.

Ad. 14: Straw: pith in cross section

Pith in cross section should be observed half way between base of ear and uppermost node. All stems of the plant should be checked and the highest score per plant recorded.

1	2	3
thin	medium	thick or filled

Ad. 15: Ear: density

The density can be assessed either visually or as measurement of the ratio of the number of spikelets/ear length.

Ad. 16: Ear: length

Length of ear should be observed excluding awns and scurs.

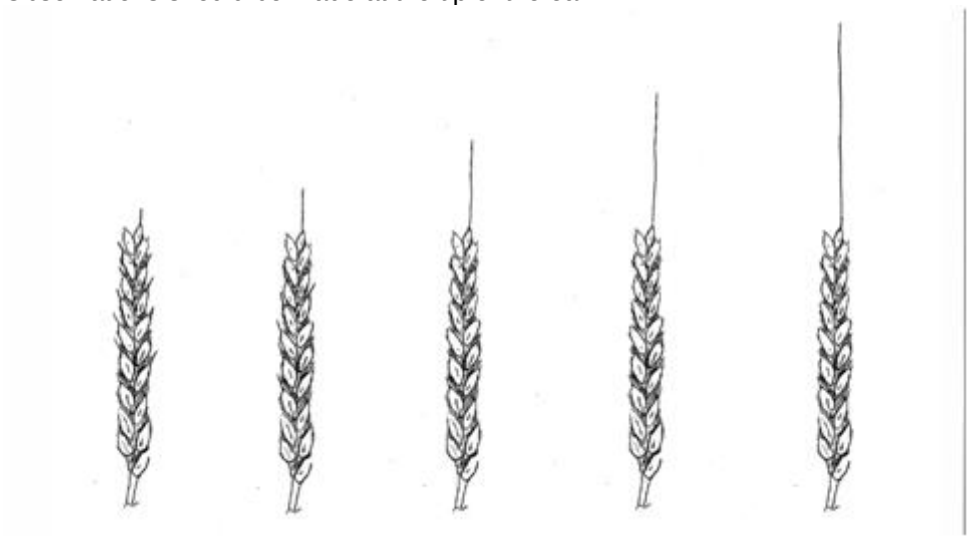
Ad. 17: Ear: scurs or awns

Observations should be made at the tip of the ear.

1	2	3
both absent	scurs present	awns present

Ad. 18: Ear: length of scurs or awns

Cannot be observed on varieties with no scurs nor awns.
Observations should be made at the tip of the ear.



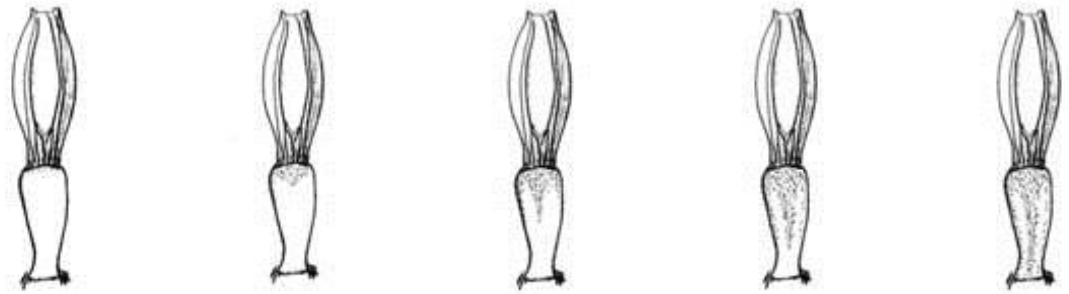
Ad. 19: Ear: color

White ear varieties may be slightly colored due to environmental conditions.

Ad. 20: Ear: shape in profile

1 tapering | 2 parallel sided | 3 slightly clavate | 4 strongly clavate | 5 fusiform

Ad. 21: Apical rachis segment: area of hairiness on convex surface



1 absent or very small | 3 small | 5 medium | 7 large | 9 very large

Ad. 22: Lower glume: shoulder width

1 absent or very narrow | 3 narrow | 5 medium | 7 broad | 9 very broad

Ad. 23: Lower glume: shoulder shape

1	3	5	7	9
strongly sloping	slightly sloping	horizontal	slightly elevated	strongly elevated

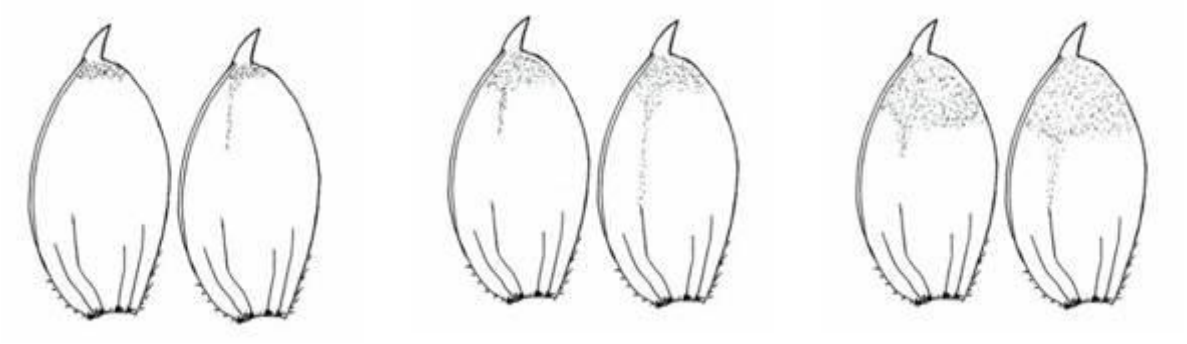
Ad. 24: Lower glume: length of beak

1	3	5	7	9
very short	short	medium	long	very long

Ad. 25: Lower glume: shape of beak

1	3	5	7	9
straight	slightly curved	moderately curved	strongly curved	geniculate

Ad. 26: Lower glume: area of hairiness on internal surface



1	3	5
very small	medium	very large

Ad. 27: 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 its description, candidate varieties can be described. At the time when the latest spring type variety is fully mature (stage 91/92 of the Zadoks decimal code) 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 (as a rule they 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 The descriptions of the growth stages of the Zadoks decimal code for cereals

Zadoks Decimal code	**Description**
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	-
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

PAYNE, P.I., and LAWRENCE, G.J., 1983. Cataloge of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for the high-molecular-weight subunits of the glutenin in hexaploid wheat. Cereal Res. Commun., 11: 29-35. 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

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 aestivum* L. emend. Fiori et Paol.
1.2	Common name	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)

(……..……..…) x (……..……..…)

female parent male parent

(b) partially known cross [ ]

(please state known parent variety(ies))

(……..……..…) x (……..……..…)

female parent male parent

(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.

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

(a) Self-pollination [ ]
(b) Hybrid [ ]
(c) Other (please provide details) [ ]

4.2.2 Other [ ]
(Please provide details)

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

(……..……..…) x (……..……..…)

female parent male parent

Three-Way Hybrid

(……..……..…) x (……..……..…)

female parent male parent

(……..……..…) x (……..……..…)

single hybrid used as female parent 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 (7)	**Time of ear emergence**		
	very early	Badiel (s), Maxwell (w)	1 [ ]
	early	Sensas (s), Solehio (w)	3 [ ]
	medium	Granary (s), Sertori (w)	5 [ ]
	late	Rosario (w), Triso (s)	7 [ ]
	very late	Adequat (w)	9 [ ]
5.2 (12)	**Lower glume: hairiness on external surface**		
	absent	Soissons (w), Triso (s)	1 [ ]
	present	Franz (w), Galera (s)	9 [ ]
5.3 (13)	**Plant: length**		
	very short	Fronton (w)	1 [ ]
	short	Apache (w), Lennox (s)	3 [ ]
	medium	FD 1 24 (s), Solehio (w)	5 [ ]
	long	Antonius (w)	7 [ ]
	very long	Capo (w)	9 [ ]
5.4 (14)	**Straw: pith in cross section**		
	thin	FD 1 24 (s), SY Moisson (w)	1 [ ]
	medium	Apache (w), KWS Flint (s)	2 [ ]
	thick or filled	Olivart (s), Synchro (w)	3 [ ]
5.5 (17)	**Ear: scurs or awns**		
	both absent	Gorda (s)	1 [ ]
	scurs present	Apache (w), Granary (s)	2 [ ]
	awns present	Sensas (s), Solehio (w)	3 [ ]
5.6 (19)	**Ear: color**		
	white	Granary (s), Solehio (w)	1 [ ]
	colored	Bastian (s), Sertori (w)	2 [ ]
5.7 (27)	**Seasonal type**		
	winter type	Solehio (w)	1 [ ]
	alternative type	SY Moisson (w)	2 [ ]
	spring type	Lennox (s)	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	*Time of ear emergence*	*late*	*early to medium*

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

Authorities may allow certain of this information to be provided in a confidential section of 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.

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

[Annex follows]

Annex

ELECTROPHORESIS

Part I

Introduction

The following Annex contains a list of characteristics derived by using electrophoresis and a description of the method to be used. UPOV decided to place these characteristics in an Annex to the Test Guidelines, thereby creating a special category of characteristic, because the majority of the UPOV members is of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis. Such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should not be used as a routine characteristic but at the request or with the agreement of the applicant of the candidate variety.

For the analysis of high molecular weight (HMW) glutenins, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS PAGE) should be used. Any alternative method may be used if it gives the same results. Glutenins are encoded by three compound loci, known as Glu-A1, Glu-B1 and Glu-D1 on the long arms of the group 1 chromosomes (Payne, 1987). 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 (see Chapter IX, Literature). The corresponding letters and apparent molecular weights are reproduced in the description of the method used.

Part II

Characteristics Derived by Using Electrophoresis

	Characteristics Caractères Merkmale	Stage1) Stade1) Stadium1)	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note
. (+)	Glutenin composition: allele expression at locus Glu-A1		band 1	bande 1	Bande 1	Meister	1
			band 2*	bande 2*	Bande 2*	Sonett, Spontan	2
	Gluténine: expression de l'allèle occupant le locus Glu-A1 Glutenin-Zusammensetzung: Allel-Ausprägung im Locus Glu-A1		no band	pas de bande	keine Bande	JB Asano	3
. (+)	Glutenin composition: allele expression at locus Glu-B1		bands 6 + 8	bandes 6 + 8	Banden 6 + 8	Meister	1
			bands 7 + 8	bandes 7 + 8	Banden 7 + 8	KWS Loft	2
	Composition de la gluténine: expression de l'allèle occupant le locus Glu-B1 Glutenin-Zusammensetzung: Allel-Ausprägung im Locus Glu-B1		bands 7 + 9	bandes 7 + 9	Banden 7 + 9	Tobak	3
			band 7 (or 7 + 9 in the presence of bands 5 + 10 of char. Glu-D1)	bande 7 (ou 7 + 9 en présence des bandes 5 + 10 du car. Glu-D1)	Bande 7 (oder 7 + 9 in Gegenwart der Banden 5 + 10 des Merkm. Glu-D1)	JB Asano	4
			bands 13 + 16	bandes 13 + 16	Banden 13+ 16	Fanion, Ronsard	5
			bands 14 + 15	bandes 14 + 15	Banden 14 + 15	Atomic	6
			bands 17 + 18	bandes 17 + 18	Banden 17 + 18	Tabasco	7
			band 20	bande 20	Bande 20	Ilias	8
			bands 6.1 + 22	bandes 6.1 + 22	Banden 6.1 + 22	-	9

. (+)	Glutenin composition: allele expression at locus Glu-D1	bands 2 + 12	bandes 2 + 12	Banden 2 + 12	Tobak	1
		bands 3 + 12	bandes 3 + 12	Banden 3 + 12	Matrix	2
	Composition de la gluténine: expression de l'allèle occupant le locus Glu-D1	bands 4 + 12	bandes 4 + 12	Banden 4 + 12	-	3
		bands 5 + 10	bandes 5 + 10	Banden 5 + 10	JB Asano	4
	Glutenin-Zusammensetzung: Allel-Ausprägung im Locus Glu-D1					

Part III

Description of the Method to be Used

Glutenin composition: allele expression at loci Glu-A1, Glu-B1 and Glu-D1

SDS PAGE Method for Analysis of HMW Glutenins from T. aestivum

1. 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.

2. 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)

3. Solutions

3.1 Extraction solution

3.1.1 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.

3.1.2 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.

3.2 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.

3.3 Gel preparation solutions

3.3.1 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 l with distilled water. This buffer can be stored at 4°C for 2 months.

3.3.2 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 l with distilled water. This buffer can be stored at 4°C for 2 months.

3.3.3 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.

3.3.4 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.

3.3.5 Stock acrylamide solution

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

3.3.6 Stock bisacrylamide solution

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

3.4 Staining solutions

3.4.1 0.25g Coomassie Brilliant Blue G-250 plus 0.75g Coomassie Brilliant Blue R-250, made up to 100 ml with water.

3.4.2 55g TCA, 65 ml glacial acetic acid, 180 ml methanol or ethanol plus 25 ml solution 3.4.1, made up to 11 with distilled water.

4. Procedure

4.1 Protein extraction

4.1.1 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.

4.1.2 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 ml is usually sufficient.

4.2 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.

4.2.1 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 ml 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.

4.2.2 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 m 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.

4.2.3 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 ml 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).

4.3 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.

4.4 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.

5 Recognition of Glutenin Alleles

This Table is designed to illustrate the molecular weight of all of the glutenin bands from each locus.

Sub-Units of HMW Glutenins: nomenclature of the individual bands

Band number	Molecular weight (kDa)
1	113
2	108
2*	108
3	107
4	106
5	105
6	100
6.1	99
7	98
8	86
9	83
10	83
12	80
13	94
14	94
15	91
16	90
17	89.5
18	89.5
20	
22	87

Note: Certain bands (e.g. bands 9 and 10) have similar molecular weights. This leads to the fact that in the presence of bands 5 + 10 of characteristic Glu-D1 two states of expression of characteristic Glu-B1, band 7 and bands 7 + 9, cannot be differentiated from one another. Therefore, in the presence of bands 5 + 10 of characteristic Glu-D1, note 4 of characteristic Glu-B1 could be either band 7 or bands 7 + 9. Other bands having similar molecular weights can be differentiated from one another by their known association with other bands. For characteristic Glu-B1, band 13 is always associated with band 16 and band 14 with band 15 while band 20 remains alone.

[End of Annex]