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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

BARLEY

UPOV Code(s):

HORDE_VUL

Hordeum vulgare L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from Germany
to be considered by the
Technical Working Party for Agricultural Crops
at its forty-sixth session, to be held in Hanover, Germany,
from 2017-06-19 to 2017-06-23

Disclaimer: this document does not represent UPOV policies or guidance

Alternative names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Hordeum vulgare L.	Barley	Orge	Gerste	Cebada

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.

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Hordeum vulgare* L..

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 and ears (if requested).

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

Seed: 3 kg
Ears: 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 ears 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 assessment of the characteristic “Seasonal type” should be carried out on at least 300 plants.

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

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 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/ear rows
B: sample size of 2000 plants

4.2.5 For the assessment of uniformity in a sample of 2000 plants, the following standards should be applied
For self-pollinated varieties a population standard of 0.1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 2000 plants, 5 off-types are allowed.
For male sterile lines a population standard of 0.2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 2000 plants, 8 off-types are allowed.
For male sterile single cross hybrids used as parent in a 3-way-hybrid a population standard of 0.5% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 2000 plants, 15 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 1, 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) Lowest leaves: hairiness of leaf sheath (characteristic 3)
(b) Ear: number of rows (characteristic 13)
(c) Ear: development of sterile spikelets (characteristic 14)
(d) Grain: rachilla hair type (characteristic 23)
(e) Grain: type (characteristic 25)
(f) Grain: hairiness of ventral furrow (characteristic 26)
(g) 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.

The varieties are indicated as follows:
(S) - spring barley
(W) - winter barley.

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 8.1

6 Not applicable

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

A: sample size of 100 plants/parts of plants/ear rows
B: sample size of 2000 plants

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**			
		Kernel: color of aleurone layer					
		whitish				(W) California, (S) Grace	1
		light grey blue				(W) SY Leoo, (S) Henley	2
		dark grey blue				(W) Saffron, (S) ---	3
		purple					4
		black					5
2.	**(*)**	**QN VG|B**	**(+)**	**25-29**			
		Plant: growth habit					
		erect				(W) ---, (S) ---	1
		semi-erect				(S) Pirona, (W) ---	3
		intermediate				(W) California, (S) Grace	5
		semi-prostate				(W) KWS Joy, (S) Quench	7
		prostate				(W) ---, (S) ---	9
3.	**(*)**	**QL VG|A**		**25-29**			
		Lowest leaves: hairiness of leaf sheath					
		absent				(W) California, (S) Grace	1
		present				(S) ---, (W) Henriette	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
4.	**(*)**	**QN VG|B**		**45-49**			
		Flag leaf: intensity of anthocyanin coloration of auricles					
		absent or very weak				(W) California, (S) ---	1
		weak				(S) Pirona, (W) ---	3
		medium				(W) SY Leoo, (S) Conchita	5
		strong				(S) Grace, (W) Semper	7
		very strong				(S) ---, (W) Meseta	9
5.		**QN VG|B**	**(+)**	**49-51**			
		Flag leaf: attitude					
		erect				(S) ---, (W) Hobbit	1
		semi-erect				(W) California, (S) Natasia	3
		horizontal				(W) Saffron, (S) Quench	5
		semi-drooping				(W) Matros, (S) Arcadia	7
		drooping				(S) ---, (W) Augusta	9
6.	**(*)**	**QN MG|B**	**(+)**				
		Time of ear emergence					
		very early				(W) ---, (S) ---	1
		early				(W) Meseta, (S) Lilly	3
		medium				(W) California, (S) Natasia	5
		late				(W) Saffron, (S) ---	7
		very late				(W) ---, (S) ---	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
7.		**QN VG|B**		**50-60**			
		Flag leaf: glaucosity of sheath					
		absent or very weak				(W) ---, (S) ---	1
		weak				(S) ---, (W) Barbara	3
		medium				(W) Saffron, (S) Pirona	5
		strong				(W) California, (S) Grace	7
		very strong				(S) ---, (W) Henriette	9
8.	**(*)**	**QN VG|B**		**60-65**			
		Awns: intensity of anthocyanin coloration of tips					
		absent or very weak				(W) California, (S) ---	1
		weak				(S) Pirona, (W) Lomerit	3
		medium				(W) Marielle, (S) Ebson	5
		strong				(S) Grace, (W) Semper	7
		very strong				(W) ---, (S) Wilma	9
9.	**(*)**	**QN VG|B**		**65-75**			
		Ear: glaucosity					
		absent or very weak				(W) Henriette, (S) Sunshine	1
		weak				(W) Matros, (S) Michelle	3
		medium				(W) Semper, (S) Arcadia	5
		strong				(W) KWS Meridian, (S) Natasia	7
		very strong				(W) ---, (S) ---	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
10.		**QN VG|B**	**(+)**	**70-80**			
		Ear: attitude					
		erect				(W) ---, (S) ---	1
		semi-erect				(W) KWS Meridian, (S) Quench	3
		horizontal				(W) Saffron, (S) Grace	5
		semi-drooping				(W) Augusta, (S) Ingmar	7
		drooping				(W) ---, (S) ---	9
11.		**QN VG|B**		**80-85**			
		Grain: anthocyanin coloration of nerves of lemma					
		absent or very weak				(W) California, (S) ---	1
		weak				(W) Hobbit, (S) Chamonix	3
		medium				(W) Marielle, (S) Quench	5
		strong				(S) Grace, (W) Atenon	7
		very strong				(S) ---, (W) Matros	9
12.	**(*)**	**QN MG|B**	**(+)**	**80-92**			
		Plant: length					
		very short				(W) ---, (S) ---	1
		short				(W) Findora, (S) Frontier	3
		medium				(W) Henriette, (S) Quench	5
		long				(S) Pirona, (W) Semper	7
		very long				(W) ---, (S) ---	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
13.	**(*)**	**QL VG|B**		**80-92**			
		Ear: number of rows					
		two				(W) California, (S) Grace	1
		six				(W) Henriette, (S) Olsok	2
14.	**(*)**	**QL VG|B**	**(+)**	**80-92**			
		Ear: development of sterile spikelets					
		none or rudimentary				(W) California, (S) Grace	1
		full				(W) Casanova, (S) Quench	2
15.	**(*)**	**QN VG|B**	**(+)**	**80-92**			
		Sterile spikelet: attitude					
		parallel				(W) California, (S) Pirona	1
		parallel to divergent				(W) KWS Joy, (S) Henley	2
		divergent				(W) Casanova, (S) Quench	3
16.	**(*)**	**PQ VG|B**	**(+)**	**80-92**			
		Ear: shape					
		strongly tapering				(W) California, (S) KWS Irina	1
		slightly tapering				(W) Hobbit, (S) Arcadia	2
		parallel				(W) Semper, (S) Natasia	3
		slightly fusiform				(W) ---, (S) ---	4
		strongly fusiform				(W) ---, (S) ---	5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17.	**(*)**	**QN MS|B/VG|B**		**80-92**			
		Ear: density					
		very sparse				(W) ---, (S) ---	1
		sparse				(W) Casanova, (S) Ingmar	3
		medium				(W) KWS Meridian, (S) Quench	5
		dense				(W) Findora, (S) Belgravia	7
		very dense				(W) ---, (S) Mercada	9
18.		**QN MS|B/VG|B**	**(+)**	**80-92**			
		Ear: length					
		very short				(W) ---, (S) ---	1
		short				(W) Champagne, (S) Mercada	3
		medium				(W) Findora, (S) Quench	5
		long				(W) California, (S) Ingmar	7
		very long				(W) ---, (S) ---	9
19.	**(*)**	**QN MS|B/VG|B**	**(+)**	**80-92**			
		Awn: length					
		very short				(S) Pirona, (W) ---	1
		short				(W) KWS Meridian, (S) Marthe	3
		medium				(W) Augusta, (S) Natasia	5
		long				(W) Lomerit, (S) Quench	7
		very long				(W) ---, (S) ---	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
20.		**QN** **MG|A/MS|A/VG|A**		**92**			
		Rachis: length of first segment					
		very short				(W) ---, (S) ---	1
		short				(W) SY Leoo, (S) Quench	3
		medium				(W) KWS Meridian, (S) Natasia	5
		long				(W) California, (S) Belgravia	7
		very long				(W) ---, (S) ---	9
21.		**QN** **VG|A**	**(+)**	**92**			
		Rachis: curvature of first segment					
		absent or very weak				(W) ---, (S) ---	1
		weak				(W) Henriette, (S) KWS Aliciana	3
		medium				(W) California, (S) Henley	5
		strong				(W) KWS Joy, (S) Ingmar	7
		very strong				(W) ---, (S) ---	9
22.	**(*)**	**QN** **VG|A**	**(+)**	**92**			
		Median spikelet: length of glume and its awn relative to grain					
		shorter				(W) ---, (S) ---	1
		equal				(W) California, (S) Quench	2
		slightly longer				(S) ---, (W) Cierzo	3
		much longer				(S) ---, (W) Champagne	4

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
23.	**(*)**	**QL**	**VG|A**	**(+)**		**80-92**			
		Grain: rachilla hair type							
		short						(W) KWS Joy, (S) Quench	1
		long						(W) California, (S) Grace	2
24.		**QN**	**VG|A**	**(+)**		**80-92**			
		Grain: spiculation of inner lateral nerves of dorsal side of lemma							
		absent or very weak						(W) California, (S) Grace	1
		weak						(W) KWS Joy, (S) Chamonix	3
		medium						(W) Champagne, (S) Henley	5
		strong						(S) ---, (W) Semper	7
		very strong						(W) ---, (S) ---	9
25.	**(*)**	**QL**	**VG|A**			**92**			
		Grain: type							
		non-husked						(S) Pirona, (W) ---	1
		husked						(S) Grace, (W) Henriette	9
26.	**(*)**	**QL**	**VG|A**	**(+)**		**92**			
		Grain: hairiness of ventral furrow							
		absent						(S) Grace, (W) Henriette	1
		present						(W) Saffron, (S) ---	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
27.	**(*)**	**PQ VG**	**(+)**				
		Seasonal type					
		winter type				(S) ---, (W) Henriette	1
		alternative type				(S) ---, (W) Farandole	2
		spring type				(S) Grace, (W) Cierzo, (W) Genie	3
28.		**QN VG|B**		**25-29**			
		NEW (proposal IT): Plant: intensity of geen color - Support: FR, UK, DE. No support: FI					
		light				(S) ---, (W) Lomerit	1
		medium				(W) Henriette, (S) Conchita	2
		dark				(W) KWS Meridian, (S) Quench	3
29.		**QL VG|A**	**(+)**	**92**			
		NEW (proposal FI, UK) Lemma: shape of base					
		bevelled				(S) Grace, (W) Henriette	1
		non-bevelled				(S) Steffi, (W) Montana	2
30.		**QL VG**					
		DELETED (according IE comments): Grain: disposition of lodicules					
		frontal					1
		clasping					2

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
31.	**QN** **VG**					
	DELETED (according IE comments): Flag leaf: length					
	short					1
	medium					2
	long					3
32.	**QN** **VG**					
	DELETED (according IE comments): Flag leaf: width					
	narrow					1
	medium					2
	broad					3
33.	**QN** **VG**					
	DELETED (according IE comments): Grain: length of rachilla compared to grain length					
	short					1
	medium					2
	long					3

8. Explanations on the Table of Characteristics

8.1 Explanations for individual characteristics

Ad. 1: Kernel: color of aleurone layer

The following pictures were provided by KR. They are included here for illustration and for discussion during the meeting.
Following the recommended UPOV approach in respect of colors it is not intended to keep the photos in the final document!
Note 1 to 3 correspond to the previous states: whitish - weakly colored - strongly colored.
DE has no other varieties than 1 - 3. Accorcing to the comments SK, AT, UK, FI, ES and FR have no varieties with state 4 or 5 as well.


whitish - Hanbaek


gray or blue - Ganghocheong


purple - Boseokchal


black - Heukgwang

Ad. 2: 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-prostate	prostate

Ad. 5: Flag leaf: attitude

1	3	5	7	9
erect	semi-erect	horizontal	semi-drooping	drooping

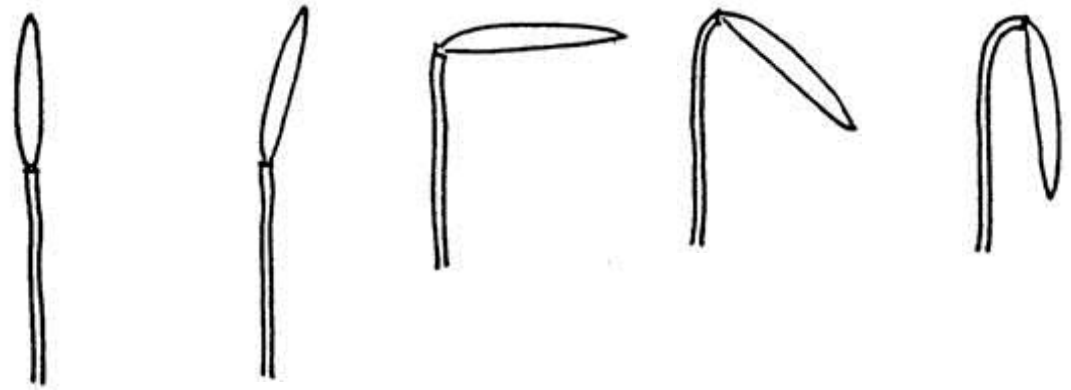
Flag leaf attitude is sensitive to the stage of plant development. Therefore, observation at the appropriate stage (stage 49–51 of the Zadoks decimal code) is of particular importance.

Flag leaf attitude relates to the angle between the main axis (stem) and the flag leaf blade. The expression of the majority of plants should be recorded without considering individual plants which may express a different attitude.

Ad. 6: Time of ear emergence

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

Ad. 10: Ear: attitude



1	3	5	7	9
erect	semi-erect	horizontal	semi-drooping	recurved

Ad. 12: Plant: length

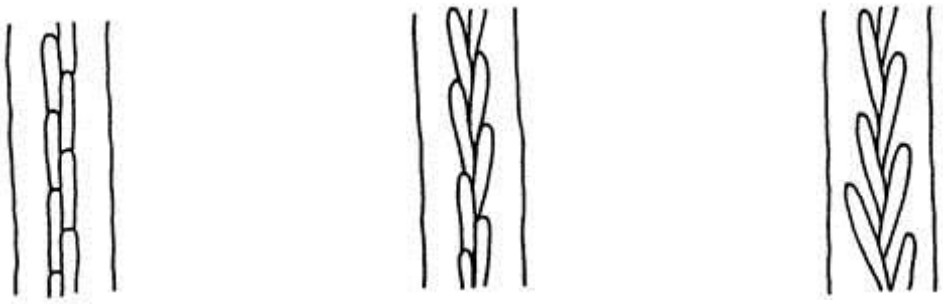
Plant length includes stem, ear and awns.

Ad. 14: Ear: development of sterile spikelets

Observation of sterile spikelet is only applicable for two row varieties.

Ad. 15: Sterile spikelet: attitude

The attitude of sterile spikelets should only be observed for varieties with fully developed spikeltes. Observations should be done in the middle third of the ear.

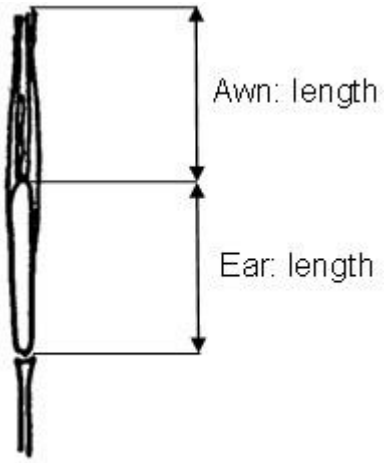


1	2	3
parallel	parallel to divergent	divergent

Ad. 16: Ear: shape

1	2	3	4	5
strongly tapering	slightly tapering	parallel	slightly fusiform	strongly fusiform

Ad. 18: Ear: length



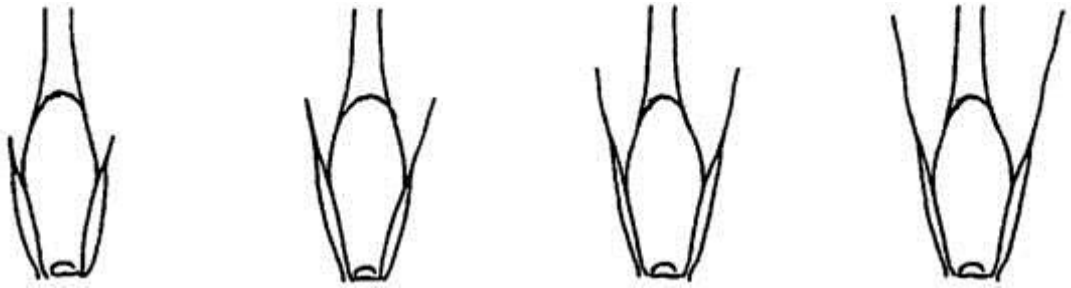
Ad. 19: Awn: length

See Ad. 18

Ad. 21: Rachis: curvature of first segment

1	3	5	7	9
absent or very weak	weak	medium	strong	very strong

Ad. 22: Median spikelet: length of glume and its awn relative to grain



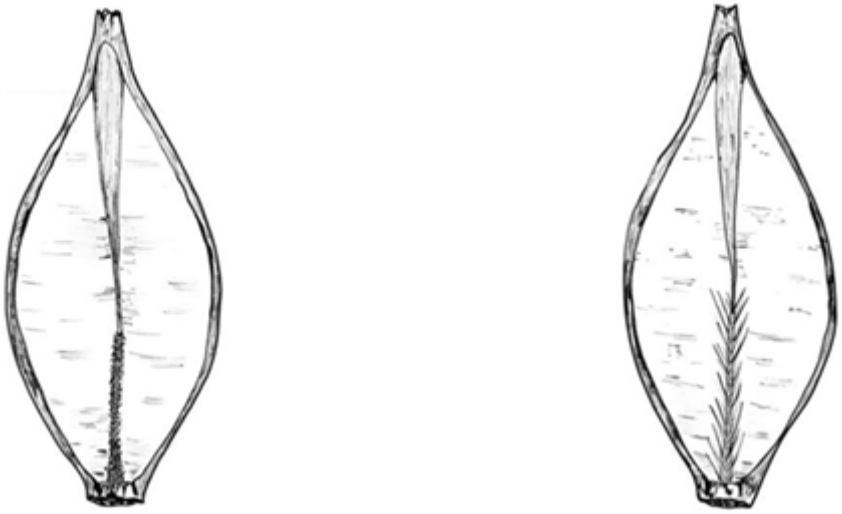
1
shorter

2
equal

3
slightly longer

4
much longer

Ad. 23: Grain: rachilla hair type



1
short

2
long

Ad. 24: Grain: spiculation of inner lateral nerves of dorsal side of lemma

none or occasionally
1 or 2 small spicules

10 or more large
regular spicules

1
absent or very weak

3
weak

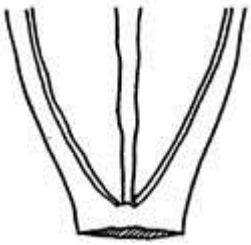
5
medium

7
strong

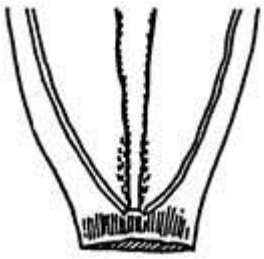
9
very strong

Ad. 26: Grain: hairiness of ventral furrow

The ventral furrow should be observed after moving the rachilla. It is of particular importance to have installed the light source at the right place. A very little number of hairs should be assessed as "present".



1
absent



9
present

Ad. 27: Seasonal type

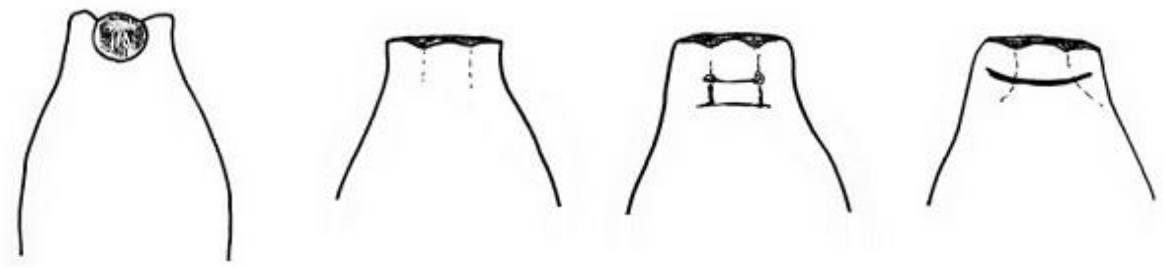
The seasonal type (need of vernalization) should be assessed on plots sown in springtime. Example varieties should always be included in the trial. When the example varieties behave according to their descriptions, the varieties under study can be described. At the time when the latest spring type variety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

1 - Winter type (high need of vernalization): The plants have reached stage 45 of the Zadoks decimal code (boots swollen) at maximum.

2 - Alternative type (partial need of vernalization): The plants have exceeded stage 45 of the Zadoks decimal code (they should normally have exceeded stage 75) and have reached stage 90 at maximum.

3 - Spring type (no need or very weak need of vernalization): The plants have exceeded stage 90 of the Zadoks decimal code.

Ad. 29: NEW (proposal FI, UK) Lemma: shape of base



1
bevelled

2
non-bevelled

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

Zadoks Decimal code	Description
	Germination
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
	Seedling growth
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
	Tillering
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
	Stem elongation
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
	Booting
41	Flag leaf sheath extending
43	Boots just visibly swollen
45	Boots swollen
47	Flag leaf sheath opening
49	First awns visible
	Inflorescence emergence
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
	Anthesis
60	Beginning on anthesis
65	Anthesis half-way
69	Anthesis completed
	Milk development
71	Caryopses watery ripe
73	Early milk
75	Medium milk
77	Late milk
	Dough development
83	Early dough
85	Soft dough
87	Hard dough
	Ripening
91	Caryopses hard (difficult to divide with thumbnail)
92	Caryopses hard (can no longer be dented with thumbnail)
93	Caryopses 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

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

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

In the case of hybrid varieties which are the subject of an application for plant breeders' rights, and where the parent lines are to be submitted as a part of the examination of the hybrid variety, this Technical Questionnaire should be completed for each of the parent lines, in addition to being completed for the hybrid variety.

1. Subject of the Technical Questionnaire

1.1 Botanical name	*Hordeum vulgare* L.
1.2 Common name	Barley

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.

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

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

(a) Self-pollination [ ]
(b) Hybrid [ ]
(c) [ ]
Please Specify

(d) 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 (3)	**Lowest leaves: hairiness of leaf sheath**		
	absent	(S) Grace, (W) California	1 [ ]
	present	(S) ---, (W) Henriette	9 [ ]
5.2 (6)	**Time of ear emergence**		
	very early	(S) ---, (W) ---	1 [ ]
	very early to early		2 [ ]
	early	(S) Lilly, (W) Meseta	3 [ ]
	early to medium		4 [ ]
	medium	(S) Natasia, (W) California	5 [ ]
	medium to late		6 [ ]
	late	(S) ---, (W) Saffron	7 [ ]
	late to very late		8 [ ]
	very late	(S) ---, (W) ---	9 [ ]
5.3 (8)	**Awns: intensity of anthocyanin coloration of tips**		
	absent or very weak	(S) ---, (W) California	1 [ ]
	weak	(S) Pirona, (W) Lomerit	3 [ ]
	medium	(S) Ebson, (W) Marielle	5 [ ]
	strong	(S) Grace, (W) Semper	7 [ ]
	very strong	(S) Wilma, (W) ---	9 [ ]
5.4 (12)	**Plant: length**		
	very short	(S) ---, (W) ---	1 [ ]
	very short to short		2 [ ]
	short	(S) Frontier, (W) Findora	3 [ ]
	short to medium		4 [ ]
	medium	(S) Quench, (W) Henriette	5 [ ]
	medium to long		6 [ ]
	long	(S) Pirona, (W) Semper	7 [ ]
	long to very long		8 [ ]
	very long	(S) ---, (W) ---	9 [ ]

	Characteristics	Example Varieties	Note
5.5 (13)	**Ear: number of rows**		
	two	(S) Grace, (W) California	1 [ ]
	six	(S) Olsok, (W) Henriette	2 [ ]
5.6 (14)	**Ear: development of sterile spikelets**		
	none or rudimentary	(S) Grace, (W) California	1 [ ]
	full	(S) Quench, (W) Casanova	2 [ ]
5.7 (23)	**Grain: rachilla hair type**		
	short	(S) Quench, (W) KWS Joy	1 [ ]
	long	(S) Grace, (W) California	2 [ ]
5.8 (25)	**Grain: type**		
	non-husked	(S) Pirona, (W) ---	1 [ ]
	husked	(S) Grace, (W) Henriette	9 [ ]
5.9 (26)	**Grain: hairiness of ventral furrow**		
	absent	(S) Grace, (W) Henriette	1 [ ]
	present	(S) ---, (W) Saffron	9 [ ]
5.10 (27)	**Seasonal type**		
	winter type	(S) ---, (W) Henriette	1 [ ]
	alternative type	(S) ---, (W) Farandole	2 [ ]
	spring type	(S) Grace, (W) Cierzo, (W) Genie	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: glaucosity*	*weak*	*medium to strong*

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.

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

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

Additional Useful Explanations

TABLE OF CONTENTS

Part I

Introduction

The following Annex contains a list of characteristics based on isozyme markers revealed by 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 based on isozyme markers revealed by 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 hordeins; polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS PAGE) is recommended. Hordeins are encoded by three compound loci known as Hor-1, Hor-2 and Hor-3 located on chromosome 5 (Hor-1 and Hor-2 on the short arm, Hor-3 on the long arm). There are a number of alleles at each locus and the analysis of hordeins 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 loci encode different groups of electrophoretically separable proteins, known as B-; C- and D-hordeins in decreasing order of mobility. The alleles at each locus can be designated by letters or numbers, or a combination of both. The relative electrophoretic mobilities (REMs) of each of the bands can also be determined.

If only C-(Hor-1) and B-(Hor-2) hordeins are of interest, then the standard reference acid PAGE method of the International Seed Testing Association (ISTA) could be used.

Part II

Characteristics derived by Protein Polymorphism

The following table indicates the REM values of the main bands present in the B-, C-.and D-hordein alleles analyzed with the SDS PAGE method and the Acid PAGE method. In comparing both methods, it should be noted that the example varieties and notes given for the individual states of expression are identical in both methods.

	Characteristics Band position in SDS PAGE method	Band position in Acid PAGE method	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
29.	**QL VG**			
	D-Hordein composition: **allele expression at locus Hor-3**			
	band 34		(W) California	1
	band 33		(W) Medina	2
	band 35		(W) Saturn	3
	band 32.5		(W) Iris	4
	band 32		(W) Princesse	5
30.	**QL VG**			
	C-Hordein composition: **allele expression at locus Hor-1**			
	bands 62+65+68	bands 27+30+32+37+39	(W) California	1
	bands 62+65+66+68	bands 27+30+32+34+37+39	(W) Lomerit	2
	bands 65+68	bands 27+30+32+37	(W) Medina	3
	bands 66.5+71	bands 32+37+41	(W) Sandra	4
	bands 61.5+66.5+71	bands 27+30+32+37+39+41	(S) Meltan	5
	bands 65	bands 32+37+38	(S) Armada	6
	bands 60 +67.5+68.5	bands 35+38	(W) Roseval	7
	bands 61+65+68+73	bands 32+37+39+41	(W) Semper	8
	bands 60+69+72	bands 38+41+42	(S) Sydney	9
	bands 64+66.5	bands 30+32+37	(W) Saturn	10
	bands 67+71	bands 34+37	(S) Pastello	11
	bands 65+68+69+70	bands 34+39+41+42	(W) Albacete	12
	bands 61.5+68+71	bands 31+34+37+38+41	(W) Borwina	13
	bands 65+67.5	bands 32+37+41+43	(W) Kendo	14
	bands 65.5+70.5		(W) Delita	15
	bands 66+70.5		(W) Maybrit	16
31.	**QL VG**			
	B-Hordein composition: **allele expression at locus Hor-2**			
	bands 79+86+88+100	bands 71+79+83+86+94+100	(S) Quench	1
	bands 79+88+91+95+97+101	bands 71+82+89+100	(S) Overture	2
	bands 79+91+92+95+97+101	bands 76+82+83+86+100	(S) Hellana	3
	bands 75+82+87+91+97	bands 66+71+76+86+93+100	(W) Caribic	4
	bands 79+86+88+97+101	bands 71+78+79+90+94	(W) Piroline	5
	bands 78+84+95+101	bands 76+81+94	(W) Ingmar	6
	bands 79+90+91+94+100	bands 71+72+75+82+85+86+100	(S) Sebastian	7
	bands 78+86+91+95+100	bands 72+76+79+90+94	(W) Sandra	8
	bands 79+82+88+91+92+100	bands 71+76+79+86	(S) Ebson	9
	bands 76+79+86+88+100	bands 71+78+83+86+94+100	(S) Trebon	10
	bands 79+86+89+92+95+101	bands 71+79+83+86+90	(W) Sigma	11
	bands 79+95+101	bands 71+76+79	(W) Midas	12

bands 78+89+92+101	bands 71+89	(W) Lomerit	13
bands 75+78+79+81+89+101	bands 79+83+86+90	(W) Findora	14
bands 75+78+79+81+83+86+88+94+95+100	bands 67+69+71+72+78+79+85+89+94	(W) Caresse	15
bands 81+84+88+90+101	bands 71+79+83+88+94	(W) Reseda	16
bands 75+78+79+81+83+86	bands 69+76+79+83+93	(W) Baronesse	17
bands 82+88+100	bands 71+72+79+85+86+91+100	(W) Albacete	18
bands 81+100	bands 72+76+100	(S) Basic	19
bands 75+79+83+89+91	bands 61+71+76+79+83	(W) Camargue	20
bands 79+84+92	bands 76+81+94+100	---	21
bands 79+91+92		(W) Libelle	22
bands 75+79+91+92+95+97+101		(W) Anja	23
bands 75+79+90+94+99		(W) Hiberna	24
bands 79+(83-85)+(89-91)+(94-96) +102		(W) Jerka	25

Part III

Description of the Method to be used

1. SDS PAGE Method for Analysis of Hordeins from *Hordeum vulgare*

1.1 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.

1.2. 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
Glycerol (d = 1.256)
Methanol
Coomassie Brilliant Blue R-250 (or equivalent)
Coomassie Brilliant Blue G-250 (or equivalent)

1.3 Solutions

1.3.1 Extraction solution

Stock solution:
6.25 ml 1M TRIS HCl buffer; PH 6.8 (see 1.3.3.2)
12.05 ml distilled water
2g SDS
10 mg Pyronin
10 ml glycerol
This solution can be stored for 2 months at 4°C.

Immediately before use; extraction solution is prepared as follows:

28.33 ml stock buffer solution plus 7.91 ml 2-mercaptoethanol made up to 100 ml with distilled water. This solution must be prepared immediately prior to use and cannot be stored.

1.3.2 Electrophoresis (running) buffer

Stock solution:
141.1 g glycine
30.0 g TRIS
10.0 g SDS
made up to 1 liter 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.

1.3.3 Gel preparation solutions

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

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

1.3.3.3 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.

1.3.3.4 1% (w/v) ammonium persulphate solution

1 g of APS dissolved in distilled water and made up to 10 ml. This solution must be prepared immediately prior to use.

1.3.3.5 Stock acrylamide solution

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

1.3.3.6 Stock bisacrylamide solution

0.3185g bisacrylamide made up to 130 ml with distilled water.

1.3.4 Staining solutions

1.3.4.1 0.25g Coomassie Brilliant Blue G-250 plus 0.75g Coomassie Brilliant Blue R-250; made up to 100 ml with water.

1.3.4.2 55 g TCA; 65 ml glacial acetic acid; 180 ml methanol plus 25 ml solution 1.3.4.1; made up to 1 liter with distilled water.

1.4. Procedure

1.4.1 Protein extraction

Individual seeds are ground using a hammer (or other device). Ground seed meal is mixed with diluted sample extraction buffer (1.3.1) in a 3 ml polypropylene hemolyse or similar tube with a screw-on 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 18,000 g for 5 minutes.

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.

1.4.2 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.

1.4.2.1 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 (1.3.3.5)
26 ml stock bisacrylamide solution (1.3.3.6)
30 ml stock gel buffer (1.3.3.1).

These should be at 4°C. The mixture is de-gassed in a 100 ml Buchner flask for 10 minutes. To this is added:
2 ml APS (1.3.3.4);
0.8 ml SDS (1.3.3.3);
40 µl TEMED (use straight from bottle).

The gels are then carefully poured; avoiding the formation of air bubbles; and polymerisation is 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 polymerisation is finished (about 30 min); the gel surface is carefully rinsed with distilled water and dried with filter paper.

1.4.2.2 Stacking gel (3.5% acrylamide; pH 6.8)

In a 50 ml Buchner flask; mix:
1.35 ml stock acrylamide solution (1.3.3.5);
3.17 ml stock bisacrylamide solution (1.3.3.6)
2.50 ml stock gel buffer (1.3.3.2) and
12.30 ml distilled water.

Following de-gassing add:
0.875 ml APS (1.3.3.4);
0.233 ml SDS (1.3.3.3);
17.5 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 polymerise for about 2 hours. The "combs" are then removed carefully from the gel cassettes and the wells rinsed using diluted electrophoresis running buffer (1.3.2).

1.4.3 Electrophoresis

The tank is filled with the appropriate volume of running buffer (1.3.2); cooled to 15 °C. Following sample loading; electrophoresis is carried out at a constant current of 8 mA/sq cm (cross-sectional area) of gel until the pyronin G has moved through the stacking gel; and then at 16 mA/sq cm of gel (maximum voltage 300V) until the marker is at the bottom of the gel. The temperature should be maintained at 15 °C.

1.4.4 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 (1.3.4.2) at room temperature. Distaining 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.

1.5 Recognition of Hordein Alleles (SDS PAGE)

The band pattern presented in the tables for B-; C- and D-hordeins are schematic and differences in band intensity have been ignored in the presentation.

B-; C- and D-hordeins: nomenclature of the individual bands and recognition of the corresponding alleles (SDS-PAGE)

Characteristic 29: **D-Hordein composition: allele expression at locus Hor-3**

Band	Example California	Note 1	2	3	4	5
32						--
32.5					--	
33			--			
34	--	--				
35				--		

Characteristic 30: C-Hordein composition: allele expression at locus Hor-1

Band	Example California	Note 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Band
60								--										60
61									--									61
61.5						--								--				61.5
62	--	--	--															62
64											--							64
65	--	--	--	--			--		--				--		--			65
65.5																--		65.5
66			--														--	66
66.5					--	--					--							66.5
67												--						67
67.5								--							--			67.5
68	--	--	--	--					--				--	--				68
68.5								--										68.5
69										--			--					69
70													--					70
70.5																--	--	70.5
71					--	--						--		--				71
72										--								72
73									--									73

Characteristic 31: B-Hordein composition: allele expression at locus Hor-2

Band	Example	Note																								Band
	Quench	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
75					--										--	--		--			--			--	--		75
76											--																76
78							--		--					--	--	--		--									78
79	--	--	--	--		--		--		--	--	--	--		--	--		--			--	--	--	--	--	--	79
81															--	--	--	--		--							81
82					--														--								82
83																--		--			--					--	83
84							--										--					--				--	84
85																										--	85
86	--	--				--			--		--	--				--		--									86
87					--																						87
88	--	--	--			--				--	--					--	--		--								88
89												--		--	--						--					--	89
90								--									--								--	--	90
91			--	--	--			--	--	--											--		--	--		--	91
92				--						--		--		--								--	--	--			92
94								--								--									--	--	94
95			--	--			--		--			--	--			--								--		--	95
96																										--	96
97			--	--	--	--																		--			97
99																									--		99
100	--	--						--	--		--					--			--	--							100
101			--	--		--	--			--		--	--	--	--		--							--			101
102																										--	102

2. Acid PAGE Method for Analysis of B- and C-Hordeins from *Hordeum vulgare*

If only B- and C-hordeins are of interest; then acid PAGE can be used. The following method is the standard reference method recommended by the International Seed Testing Association.

2.1. Apparatus and Equipment

Various designs of vertical electrophoresis equipment have been used successfully; including those available from Biometra; Bio-Rad; Desaga and Pharmacia-LKB. The power supply used should be capable of operating at constant voltage and constant current.

2.2. Chemicals

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

Acrylamide ("specially purified for electrophoresis")
Bisacrylamide ("specially purified for electrophoresis")
Urea
Glacial acetic acid
Glycine
Ferrous sulphate
Ascorbic acid
Hydrogen peroxide
Monothioglycerol
Pyronin G
Trichloroacetic acid (TCA)
Methanol
2-chloroethanol
Coomassie Brilliant Blue G-250 (or equivalent)
Coomassie Brilliant Blue R-250 (or equivalent)

2.3. Solutions

2.3.1 Extraction solution

Pyronin G (0.05%) (w/v) in 2-chloroethanol (20%) (v/v) containing urea (18% w/v) and monothioglycerol (1% v/v) (keep cold or prepare fresh).

2.3.2 Tank buffer solution

Glacial acetic acid (4 ml) and glycine (0.4g); made up to 1 litre with distilled water; keep cold.

2.3.3 Gel buffer solution

Glacial acetic acid (20 ml) and glycine (1.0g); made up to 1 litre with distilled water; keep cold.

2.3.4 Staining solutions

0.25g Coomasie Brilliant Blue G-250 + 0.75g Coomassie Brilliant Blue R-250 in 100 ml water.

55g TCA; 65 ml glacial acetic acid; 180 ml methanol; plus 25 ml solution 2.3.4.1; made up to 1 litre with distilled water.

2.4. Procedure

2.4.1 Protein extraction

Single seeds are crushed with pliers or by similar means and transferred to 1.5 ml polypropylene centrifuge tubes or to micro-titer plates. Extraction solution (2.3.1) (0.3 ml) is added and the tubes or plates are allowed to stand overnight at room temperature. If necessary; the tubes are centrifuged at 18,000xg and the supernatants used for electrophoresis.

2.4.2 Preparation of the gel

Clean and dry gel cassettes are assembled; according to the design of the equipment. Treating the glass plates with silicon prior to assembly can facilitate subsequent removal of the gel. The gel cassettes can incorporate a plastic backing sheet (e.g. "Gel Bond PAG"; FMC Corporation). This supports the gel during subsequent operations. To make 100 ml of gel medium; gel buffer at 4°C (2.3.3) (approximately 60 ml) is taken and the following added: acrylamide (10g); bisacrylamide (0.4g); urea (6g); ascorbic acid (0.1g); ferrous sulphate (0.005g). The solution is stirred and made up to 100 ml with cold (4°C) stock gel buffer solution (2.3.3). Freshly prepared 0.6% (v/v) hydrogen peroxide solution (0.35 ml per 100 ml of gel medium) is added; mixed quickly and the gel poured. An acrylic "comb" is placed in the top of the cassette; to make wells in the gel. Polymerisation is carried out at room temperature and should be complete in five to 15 minutes. If not; it may be necessary to adjust the volume of hydrogen peroxide added. The gel mixture should over-fill the cassette; or be over-layed with water; to ensure satisfactory polymerisation of the upper surface.

2.4.3 Electrophoresis

The acrylic comb is removed from the gel and the sample wells washed with tank buffer (2.3.2). The tank is filled with an appropriate volume of buffer (2.3.2) (depending on the equipment used). Samples (10-20 ul) are loaded into the wells and the gel placed in the tank, ensuring that the sample wells are completely filled. The temperature of the lower buffer chamber should be kept at 15°C. Electrophoresis is carried out at a constant voltage of not more than 60V/cm^2 (cross-sectional area) of gel (which corresponds to a voltage of 500V for two gels 16 cm wide and 0.15 cm thick) for twice the time taken for the pyronin G marker to leave the gel. It must be remembered that the anode (positive electrode) is at the origin (top of the gel) in this system.

2.4.4 Fixing and staining

The gel cassette is removed from the tank, opened and the gel placed in a plastic box containing 200 ml of staining solution (2.3.4.2). Staining is carried out overnight at room temperature. Destaining if necessary is carried out by placing gels in water for about two to 3 hours at room temperature. Gels can then be dried or stored in sealed polythene bags at 4°C.

It should be noted that other procedures, such as the use of increased temperatures or the use of mixtures of TCA and Coomassie Brilliant Blue G, will give satisfactory staining of gels. The final quality control criterion, both for gel preparation and gel staining, is to analyse the suggested example varieties on each batch of gels. The separation of the designated bands, and their relative electrophoretic mobilities, must be clear and correct in order for the procedures to be satisfactory.

2.5 Recognition of Hordein Alleles (Acid PAGE)

B- and C-Hordeins: nomenclature of the individual bands and recognition of the corresponding alleles: acid PAGE

Characteristic 30: C-Hordein composition: allele expression at locus Hor-1

Band	Example California	Note 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Band
25																25
27	--	--	--	--		--										27
30	--	--	--	--		--					--					30
31														--		31
32	--	--	--	--	--	--	--		--		--				--	32
34			--									--	--	--		34
35								--								35
37	--	--	--	--	--	--	--		--		--	--		--	--	37
38							--	--		--				--		38
39	--	--	--			--			--				--			39
41					--	--			--	--			--	--	--	41
42										--			--			42
43															--	43
		Alleles according to acid PAGE nomenclature														
		10	10A	1	11	17	6	19	2	4	5	18	14	8	3	

Characteristic 31: B-Hordein composition: allele expression at locus Hor-2

Band	Example Quench	Note 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Band
61																					--		61
66					--																		66
67																--							67
69																--		--					69
71	--	--	--		--	--		--		--	--	--	--	--		--	--		--		--		71
72								--	--							--			--	--			72
75								--															75
76				--	--		--		--	--			--					--		--	--	--	76
78						--					--					--							78
79	--	--				--			--	--		--	--		--	--	--	--	--		--		79
81							--															--	81
82			--	--				--															82
83	--	--									--	--			--		--	--			--		83
85								--								--			--				85
86	--	--		--	--			--		--	--	--			--				--				86
88																	--						88
89			--											--		--							89
90						--			--			--			--								90
91																			--				91
93					--													--					93
94	--	--				--	--		--		--					--	--					--	94
97																							97
100	--	--	--	--	--			--			--								--	--		--	100
		3	4	13	14	-	9	1	7	6	-	-	11	16	-	18	-	19	8	15	12	10	

[End of Annex and of document]