CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Hordeum vulgare L. *sensu lato*

BARLEY

UPOV Species Code: HORDE_VUL

Adopted on 11/03/2010

Entered into force on 01/03/2010

I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/19/10 dated 4th November 1994 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of ***Hordeum vulgare* L. *sensu lato***.

II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the Examination Office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection of the Examination Office as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO if no plant material has been received. However, if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Seed requirements

The final dates for request for technical examination and sending of Technical Questionnaire as well as submission date of plant material by the applicant, and quantity of plant material to be supplied by the applicant are published on the CPVO website and in the S2 official gazette.

Quality of seed: ..... The minimum requirements for germination capacity, analytical purity and seed health should not be less than the standards laid down in Council Directive 66/402/EEC.
If the number of grains in 1 ear is insufficient, it is admitted to use 2 ears per plant to set up ear-rows.

Seed Treatment: ..... The plant material must not have undergone any treatment unless the CPVO and the examination office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Labelling of sample: ..... - Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination Office reference (if known)
- Name of applicant
- The phrase “On request of the CPVO”.

III CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation (EC) No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.
- in case of hybrids, all components of hybrid varieties in common knowledge must be considered as part of the reference collection.

The composition of the variety collection in each Examination Office depends on the ecological conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other offices involved in DUS-testing of barley. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The list of characteristics derived from electrophoresis as in Part II of Annex II should only be used as a complement to other differences in morphological or physiological characteristics.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation (EC) No. 1239/95, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics that could be used for grouping are the following (CPVO numbering; G for grouping in table of characteristics)

a) Lower leaves: hairiness of leaf sheaths (characteristic 2)

b) Ear: number of rows (characteristic 11)

c) Grain: rachilla hair type (characteristic 21)

d) Grain: hairiness of ventral furrow (characteristic 25)

e) Seasonal type (characteristic 28)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing cycle.

The test design is as follows:

Each test should include about 2000 plants which should be divided between two or more replicates. The assessment for the characteristic 'Seasonal type' should be carried out on at least 500 plants.

If ear rows are used, tests should be conducted on 100 ears.

In case of hybrids, the parent lines have to be included in the test and should be tested and assessed as any other self-pollinating variety. The observations on the hybrid variety itself should be made on at least 200 plants.

All observations for the assessment of distinctness on individual plants should be made on 20 plants or parts of 20 plants.

6. Special tests

In accordance with Article 83(3) of Council Regulation (EC) No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation (EC) No. 2100/94.

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

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

Qualitative characteristics:

In the case of characteristics which show discrete discontinuous states of expression, a difference between two varieties is clear if the respective characteristics have expressions which fall into two different states.

Quantitative characteristics:

Characteristics which show a continuous range of expression from one extreme to the other may be either measured or visually observed.

In the case of visually observed characteristics, a difference between two varieties is clear if the expression of the respective characteristics differs by at least the span of one note, taking into account the variability observed within the varieties.

If distinctness is assessed using the t-test least significant difference the difference between two varieties is clear if it occurs with the same sign at the 1% significance level or less ($p<0.01$) in two consecutive or two out of three growing cycles.

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p<0.01$) in a test over either two or three years.

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

b) **Uniformity**

Uniformity is assessed by visual observation and the detection of off-types.

The number of off-types in a sample size of 2000 plants or parts of plants should not exceed 5 in 2000 (Population standard of 0.1% with an acceptance probability of ≥ 95%).
Characteristics which should be observed on a sample size of 2000 plants are indicated by a “B” in the table of characteristics.

For male sterile lines, the number of off-types in a sample size of 2000 plants should not exceed 8 in 2000 (Population standard of 0.2% with an acceptance probability of ≥ 95%).

For hybrids, the number of off-types in a sample size of 200 plants should not exceed 27 in 200 (Population standard of 10% with an acceptance probability of ≥ 95%).

The number of off-types in a sample size of 100 ear-rows, plants or parts of plants should not exceed 3 in 100 (Population standard of 1% with an acceptance probability of ≥ 95%).

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

For all varieties except hybrid varieties, a re-submission of plant material may be allowed for the second growing cycle if in the first growing cycle the number of off-types did not exceed 15 plants in a sample size of 2000 plants (Population standard of 0.5% with an acceptance probability of ≥ 95%) or 9 plants, parts of plants or ear rows in a sample size of 100 (Population standard of 5% with an acceptance probability of ≥ 95%).

Characteristics which should be observed on a sample size of 100 plants are indicated by an "A" in the table of characteristics. For these "A" characteristics, the assessment of uniformity can be done in 2 steps. In a first step, 20 plants or parts of plants are observed. If no off-types are observed, the variety is declared to be uniform. If more than 3 off-types are observed, the variety is declared not to be uniform. If 1 to 3 off-types are observed, an additional sample of 80 plants or parts of plants must be observed.

With respect to the use of enzyme electrophoresis, the Office follows the actual UPOV approach as laid down in part I of Annex 3 hereto. If electrophoresis is used for testing distinctness, the same population standard and the same acceptance probability as for other characteristics should be applied. However, a sequential analysis approach could be applied to reduce the workload.

Electrophoretic characteristics with a lack of uniformity shall not be taken into account for the assessment of distinctness.

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity. Seed samples of further submissions included in any test must show the same expression of characteristics as the material originally supplied.

IV REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing cycles but in some cases three growing cycles may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the Examination Office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V LIAISON WITH THE APPLICANT

If problems arise during the course of the test the CPVO should be informed so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

VI ENTRY INTO FORCE

The present protocol enters into force on **01/03/2010**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the new TP. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for the submission of plant material for the first growing period.

In cases where the CPVO requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process of being carried out at the moment of the request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

****

ANNEXES TO FOLLOW

ANNEX I PAGE

Legend:

(+) See explanations on the Table of characteristics
G Grouping characteristic

Type of 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

00-99 Decimal Code for the Growth Stages

When a method of observation is attributed to a certain characteristic, the first differentiation is made depending if the action taken is a visual observation (V) or a measurement (M).

The second differentiation deals with the number of observations the expert attributes to each variety, thus the attribution of either G or S.

If a single observation of a group consisting of an undefined number of individual plants is appropriate to assess the expression of a variety, we talk about a visual observation or a measurement made on a group of plants, thus we attribute the letter G (either VG or MG). If the expert makes more than one observation on that group of plants, the decisive part is that we have at the end only one data entry per variety which means that we have to deal with G (e.g. measurement of plant length on a plot – MG, visual observation of green colour of leaves on a plot – VG).

If it is necessary to observe a number of individual plants to assess the expression of a variety, we should attribute the letter S (thus either VS or MS). Single plant data entries are kept per variety for further calculations like the variety mean (e.g. measurement of length of ears – MS, visual observation of growth habit of single plants in grasses – VS). The number of individual plants to be observed in such cases is stated in section III.5.

ANNEX II

ANNEX III

ANNEX I

TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Characteristics	Stage[1] Method	Examples[2]	Note
1.	**1.**	**Plant: growth habit**	25-29		
(+)[3]		erect	B; VG	-;-	1
		semi-erect		Marinka; Klaxon	3
		intermediate		Plaisant; Alexis	5
		semi-prostrate		Pastoral; Digger	7
		prostrate		Celtic; Grit	9
2.	**2.**	**Lowest leaves: hairiness of leaf sheats**	25-29		
		absent	A; VG	Marylin; Alexis	1
G		present		Pastoral; Ceres	9
3.		**Flag leaf: intensity of anthocyanin coloration of auricles**	45-49		
		absent or very weak	B; VG	Noveta; -	1
		weak		Reinette; Auto	3
		medium		Catania; Atem	5
		strong		Barberousse; Prisma	7
		very strong		Melusine; -	9
4.	**5.**	**Plant: frequency of plants with recurved flag leaves**	47-51		
(+)		absent or very low	B; VG	-; Icare	1
		low		Rebelle; Atem	3
		medium		Pastoral; Alexis	5
		high		Krimhild; Grit	7
		very high		-; -	9

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

[2] Example varieties, separated by a semicolon, are indicated for winter barley before the semicolon, for spring barley they follow the semicolon. Example varieties are given as an indication, others may be used.

[3] See explanations in Annex 1 in 'Explanations and Methods'

CPVO N°	UPOV N°	Characteristics	Stage1 Method	Examples2	Note
5.	**6.**	**Flag leaf: glucosity of sheet**	50-60		
		absent or very weak	B; VG	-; -	1
		weak		-; -	3
		medium		Brunhild; Marielle	5
		strong		Marylin; Alexis	7
		very strong		Sereia; Pompadour	9
6.	**7.**	**Time of ear emergence (first spikelet visible on 50% of ears)**	50-52		
		very early	B; MG	Sereia; -	1
		early		Barberousse; Sewa	3
		medium		Venus; Alexis	5
		late		Borwina; Canut	7
		very late		Brunhild; -	9
7.		**Awns: intensity of anthocyanin coloration of tips**	60-65		
		absent or very weak	B; VG	Monika; -	1
		weak		Rebelle; Berenice	3
		medium		Fedora; Alexis	5
		strong		Susi; Atem	7
		very strong		Frolic; Beate	9
8.	**10.**	**Ear: glucosity**	65-75		
		absent or very weak	B; VG	Caline; Auto	1
		weak		Brunhild; Grit	3
		medium		Clarine; Alexis	5
		strong		Puffin; Volga	7
		very strong		Sereia; Mette	9

CPVO N°	UPOV N°	Characteristics	Stage1 Method	Examples2	Note
9.	**11.**	**Ear: attitude**	70		
(+)		erect	B; VG	Sigra; Volga	1
		semi-erect		Jana; Digger	3
		horizontal		Jaidor; Nomad	5
		semi-recurved		Melusine; Sissi	7
		recurved		-; -	9
10.	**12.**	**Plant: length (stem; ear and awns)**	80-92		
		very short	B; MG	Fedora; Meltan	1
		short		Pastoral; Triumph	3
		medium		Rebelle; Omega	5
		long		Frances; Ida	7
		very long		-; Aura	9
11.	**13.**	**Ear: number of rows**	80-92		
		two	B; VG	Pastoral; Aramir	1
G		more than two		Rebelle; Dobla	2
12.	**14.**	**Ear: shape**	80-92		
(+)		tapering	A; VG	Intro; Prisma	3
		parallel		Rebelle; Nomad	5
		fusiform		Criter; Pamela	7
13.	**15.**	**Ear: density**	80-92		
		very lax	A; VG	-; -	1
		lax		Express; Teo	3
		medium		Susi; Alexis	5
		dense		Catinka; Pompadeur	7
		very dense		Criter; Dobla	9

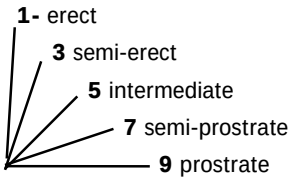
CPVO N°	UPOV N°	Characteristics	Stage1 Method	Examples2	Note
14.	**16.**	**Ear: length (excluding awns)**	80-92		
		very short	A; MS	-; -	1
		short		Krimhild; Nancy	3
		medium		Barberousse; Alexis	5
		long		Pastoral; Nomad	7
		very long		-; -	9
15.	**17.**	**Awn: length (compared to ear)**	80-92		
(+)		short	A; MS	Puffin; Menuet	3
		medium		Fiction; Nomad	5
		long		Jana; Troubadour	7
16.	**18.**	**Rachis: length of first segment**	92		
		short	A; MS	Barberousse; Triumph	3
		medium		Marinka; Volga	5
		long		Jaidor; Michka	7
17.	**19.**	**Rachis: curvature of first segment**	92		
(+)		absent or very weak	A; VG	Sigra; Prisma	1
		weak		Barberousse; Alexis	3
		medium		Pastoral; Aramir	5
		strong		Giga; Berenice	7
		very strong		-; Cameo	9
18.		**Ear: development of sterile spikelets**	92		
		non or rudimentary		Barcelona; Baroness	1
		full	A; VG	Madou; Alexis	2
19.	**20.**	**Sterile spikelet: attitude (in mid-third of ear)**	92		
(+)		parallel	A; VG	Isolde; -	1
		parallel to weakly divergent		Regina; Chariot	2
		divergent		Madou; Alexis	3

CPVO N°	UPOV N°	Characteristics	Stage1 Method	Examples2	Note
20.	**21.**	**Median spikelet: length of glume and its awn relative to grain**	92		
(+)		shorter	A; VG	Alpha; Ceres	1
		equal		Rebelle; Alexis	2
		longer		Manitou; Steffi	3
21.	**22.**	**Grain: rachilla hair type**	80-92		
(+)		short	A; VG	Barberousse; Atem	1
G		long		Pastoral; Alexis	2
22.	**23.**	**Grain: husk**	92		
		absent		Rondo; Taiga	1
		present	A; VG	Marinka; Alexis	9
23.	**24.**	**Grain: anthocyanin coloration of nerves of lemma**	80-85		
		absent or very weak	B; VG	Express; Troubadour	1
		weak		Rebelle; Prisma	3
		medium		Baraka; Lenka	5
		strong		Susi; Teo	7
		very strong		-; -	9
24.	**25.**	**Grain: spiculation of inner lateral nerves of dorsal side of lemma**	92		
(+)		absent or very weak	A; VG	Sonja; Alexis	1
		weak		Colombo; Nomad	3
		medium		Venus; Perun	5
		strong		Barberousse; Volga	7
		very strong		Noveta; -	9
25.	**26.**	**Grain: hairiness of ventral furrow**	92		
(+)		absent	A; VG	Pastoral; Alexis	1
G		present		Plaisant; Cheri	9

CPVO N°	UPOV N°	Characteristics	Stage1 Method	Examples2	Note
26.	**27.**	**Grain: disposition of lodicules**	92		
(+)		frontal		Reinette; Prisma	1
		clasping	A; VG	Rebelle; Nomad	2
27.	**28.**	**Kernel: colour of aleuron layer**	85-87		
(+)		whitish	A; VG	Express; Alexis	1
		weakly coloured		Angora; -	2
		strongly coloured		Pastoral; -	3
28.	**29.**	**Seasonal type**			
(+)		winter type	B; VG	Target; -	1
		alternative type		Novetta; -	2
G		spring type		-; Alexis	3

EXPLANATIONS AND METHODS

Ad 1: Plant: growth habit

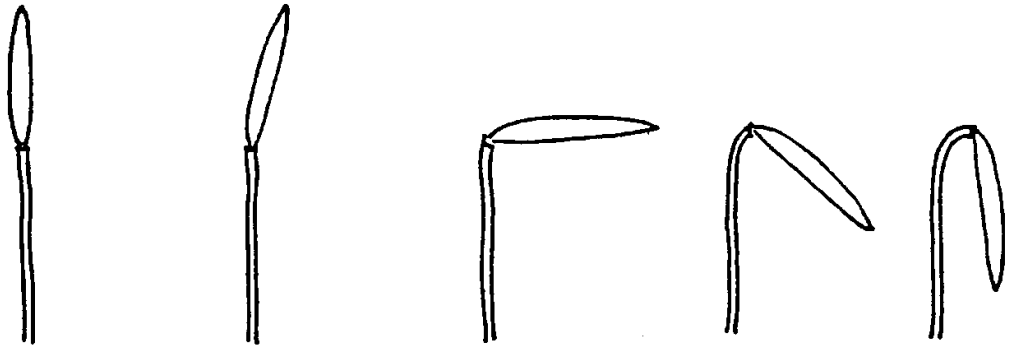


The growth habit should be assessed visually from the attitude of the leaves and tillers. The angle formed by the outer leaves and the tillers with an imaginary vertical axis should be used.

Ad 4: Plant: frequency of plants with recurved flag leaves

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

Ad 9: Ear: attitude



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

Ad 12: Ear: shape

3
tapering

5
parallel

7
fusiform

Ad 1: Awn: length compared to ear

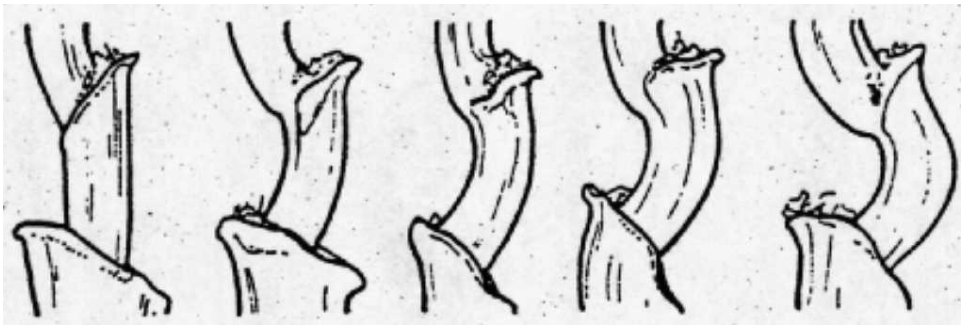
3
short

5
medium

7
long

The state "medium" means that the length of the awns is equal to that of the ear.

Ad 17: Rachis: curvature of first segment



1
absent or
very weak

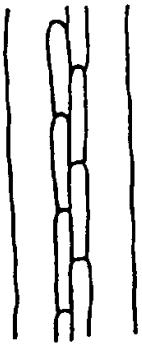
3
weak

5
medium

7
strong

9
very strong

Ad 19: Sterile spikelet: attitude (in mid-third of ear)



1
parallel

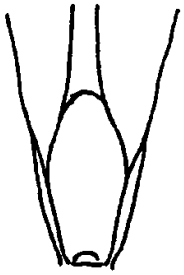
2
parallel to
weakly divergent

3
divergent

Ad 20: Median spikelet: length of glume and its awn relative to grain

1
shorter

2
equal



3
longer

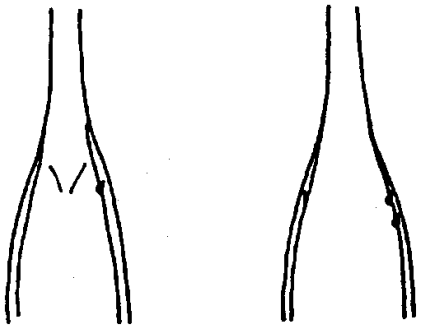
Ad 21: Grain: rachilla hair type

1
short

2
long

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

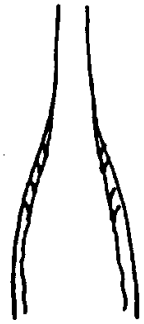
none or occasionally 1 or 2 small spicules



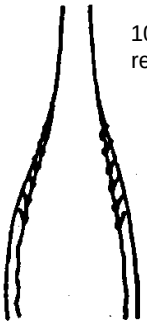
1
absent or very weak

3
weak

5
medium



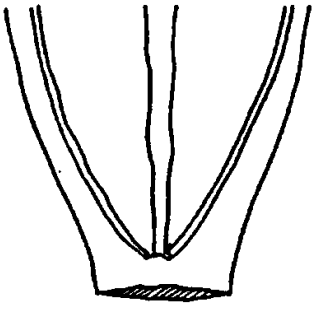
7
strong



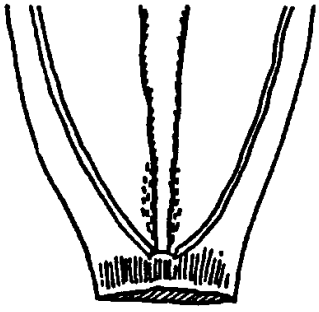
9
very strong

10 or more large regular spicules

Ad 25: Grain: hairiness of ventral furrow

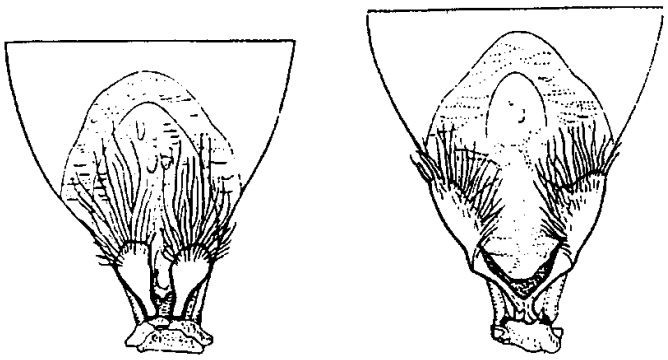


1
absent



9
present

Ad 26: Grain: disposition of lodicules



1
frontal

2
clasping

Ad 27: Kernel: colour of aleuron layer

The colour of the aleuron layer should be assessed visually after the kernel is put in the water for 12 hours. If necessary; a magnifying glass may be used.

Ad 28: Seasonal type

The seasonal type should be assessed on one or several plots sown in springtime. Example varieties should always be included in the plots. When the example varieties behave according to this description; the varieties under study can be described. At the time when the latest spring type variety is fully mature (stage 91/92 of the Eucarpia decimal code); the growth stage reached by the respective variety should be assessed. The states of expression are defined as follows:

Winter type:	The plants have reached stage 45 of the Eucarpia decimal code (boots swollen) at maximum
Alternative type:	The plants have exceeded stage 45 of the Eucarpia decimal code-as a rule they have exceeded stage 75--and have reached stage 90 at maximum
Spring type:	The plants have exceeded stage 90 of the Eucarpia decimal code.

DECIMAL CODE FOR THE GROWTH STAGE[4]

2- digit Code	General description	Feekes'Scale	Additional remarks on Wheat; Barley; Rye; Oats and Rice
	Germination		
00	Dry seed		
01	Start of inhibition		
02	-		
03	Imbibition complete		
04	-		
05	Radicle emerged from caryopsis		
06	-		
07	Coleoptile emerged from caryopsis		
08	-		
09	Leaf just at coleoptile tip		
	Seedling growth		
10	First leaf through coleoptile	1 - Second leaf visible (less than 1 cm)	
11	First leaf unfolded (1)		
12	2 leaves unfolded	50% of laminae 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	2	This section to be used to supplement records from other sections of the table: "Concurrent codes".
22	Main shoot and 2 tillers	3	
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 (2)	4-5	In rice: vegetative lag phase
31	1st node detectable	6	Jointing stage
32	2nd node detectable	7	
33	3rd node detectable		Above crown nodes
34	4th node detectable		
35	5th node detectable		
36	6th node detectable		
37	Flag leaf just visible	8	
38	-	-	Pre-boot stage
39	Flag leaf ligule / collar just visible	9	In rice: Opposite auricle

[4] Reproduced from EUCARPIA Bulletin No. 7, 1974, pp.49 - 52, with the kind permission of the authors. For further information, see J.C. Zadoks, T.T. Chang and C.F. Konzak, EUCARPIA Bulletin No. 7, 1974, pp. 42 - 52. The French translation has been kindly furnished by Mrs. R. Cassini, Mr. R. Cassini and Mr. R. Marie. The German translation has been kindly furnished by Mr. A.O. Klomp and Mrs. I. Volk.

2- digit Code	General description	Feekes'Scale		Additional remarks on Wheat; Barley; Rye; Oats and Rice
	Booting			Little enlargement of the
40	-			inflorescence; early-boot stage
41	Flag leaf sheath extending			
42	-			
43	Boots just visibly swollen			Mid-boot stage
44	-	10		
45	Boots swollen			Late-boot stage
46	-			
47	Flag leaf sheath opening			
48	-			
49	First awns visible		10.1	In awned forms only
	Inflorescence emergence			
50	First spikelet of inflorescence just	N		N = non-synchronous crops
51	visible	S		
52	1/4 of inflorescence emerged	N	10.2	
53		S		S = synchronous crops
54	1/2 of inflorescence emerged	N	10.3	
55		S		
56	3/4 of inflorescence emerged	N	10.4	
57		S		
58	Emergence of inflorescence	N	10.5	
59	completed	S		
	Anthesis			
60	Beginning of anthesis	N	10.51	
61		S		Not easily detectable in barley.
62	-			In rice: usually immediately
63	-			following heading
64	Anthesis half-way	N	10.52	
65		S		
66	-			
67	-			
68	Anthesis complete	N	10.53	
69		S		
	Milk development			
70	-			
71	Caryopsis watery ripe		10.54	
72	-			
73	Early milk			Increase in solids of liquid
74	-		11.1	endosperm notable when
75	medium milk			crushing the caryopsis between
76	-			fingers
77	Late milk			
78	-			
79	-			
	Dough development			
80	-			
81	-			
82	-			
83	Early dough			
84	-			
85	Soft dough		11.2	Fingernail impression not held
86	-			
87	Hard dough			Fingernail impression held;
88	-			inflorescence losing chlorophyll
89	-			

2- digit Code	General description	Feekes'Scale	Additional remarks on Wheat; Barley; Rye; Oats and Rice
	Ripening		
90	-		In rice: terminal spikelets ripened.
91	Caryopsis hard (difficult to divide by thumb-nail) (3)	11.3	
92	Caryopsis hard (can no longer be dented by thumb-nail) (4)	11.4	In rice: 50% of spikelets ripened
93	Caryopsis loosening in daytime		In rice: over 90% of spikelets ripened (5)
94	Over-ripe; straw dead and collapsing		
95	Seed dormant		Risk of grain loss by shedding
96	Viable seed giving 50% germination		
97	Seed not dormant		
98	Secondary dormancy induced		
99	Secondary dormancy lost		
	Transplanting and recovery (rice only)		
T1	Uprooting of seedlings		
T2	-		
T3	Rooting		
T4	-		
T5	-		
T6	-		
T7	Recovery of shoots		
T8	-		
T9	Resumption of vegetative growth		

Notes on the Table of the Decimal Code for the Growth Stages or Cereals

(1) Stage of seedling inoculation with rust in the greenhouse.

(2) Only applicable to cereals with a prostrate or semi-prostrate early growth habit.

(3) Ripeness for binder (ca. 16% water content). Chlorophyll of inflorescence largely lost.

(4) Ripeness for combine harvester (less than 16% water content).

(5) Optimum harvest time.

ANNEX II

ELECTROPHORESIS

Additional Useful Explanations

TABLE OF CONTENTS PAGE

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 member States 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 has reconfirmed 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 on the short (Hor-1 and -2) or long (Hor-3) arm of chromosome 5. 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 using electrophoresis

CPVO N°	Characteristics	Stage[5] Method	Examples[6]	Note
30.	**D-Hordein composition: allele expression at locus Hor-3**			
(+)	band 34		Atem	1
	band 33		Natalie	2
	band 35		Franka	3
	band 32.5		Iris	4
	band 32		Princesse	5
31.	**C-Hordein composition: allele expression at locus Hor-1**			
(+)	bands 62+65+68		Atem	1
	bands 62+65+66+68		Regatta	2
	bands 65+68		Pirate	3
	bands 66.5+71		Athos	4
	bands 61.5+66.5+71		Norka	5
	bands 65		Birka	6
	bands 60 +67.5+68.5		Pamela	7
	bands 61+65+68+73		Igri	8
	bands 69+72		Goelette	9
	bands 64+66.5		Catinka	10
	bands 67+71		Ombelle	11
	bands 65+68+69+70		Albacete	12
	bands 61.5+68+71		Borwina	13
	bands 65+67.5		Kendo	14
	bands 65+67.5		Kendo	14
	bands 65.5+70.5		Delita	15
	bands 66+70.5		Noveta	16

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

[6] Example varieties, separated by a semicolon, are indicated of winter barley before the semicolon, for spring barley they follow the semicolon. Example varieties are given as an indication, others may be used.

CPVO N°	Characteristics	Stage5 Method	Examples6	Note
32.	**B-Hordein composition: allele expression at locus Hor-2**			
(+)	bands 79+86+88+100		Atem	1
	bands 79+88+91+95+97+101		Aramir	2
	bands 79+91+92+95+97+101		Valerie	3
	bands 75+82+87+91+97		Carina	4
	bands 79+86+88+97+101		Piroline	5
	bands 78+84+95+101		Catinka	6
	bands 79+90+91+94+100		Regatta	7
	bands 78+86+91+95+100		Igri	8
	bands 79+82+88+91+92+101		Grit	9
	bands 76+79+86+88+100		Birka	10
	bands 79+86+89+92+95+101		Sigma	11
	bands 79+95+101		Midas	12
	bands 78+89+92+101		Criter	13
	bands 75+78+79+81+89+101		Ditta	14
	bands 75+78+79+81+83+86+88+94+95+100		Caresse	15
	bands 81+84+88+90+101		Reseda	16
	bands 75+78+79+81+83+86		Baronesse	17
	bands 82+88+100		Albacete	18
	bands 81+100		Digger	19
	bands 75+79+83+89+91		Camargue	20
	bands 79+91+92		Libelle	22
	bands 75+79+91+92+95+97+101		Triton	23
	bands 75+79+90+94+99		Hiberna	24
	bands 79+(83-85)+(89-91)+(94-96)+102		Jerka	25

Part III

Description of the method to be used

Hordein composition: allele expression at loci Hor-3(30); Hor-1(31) and Hor-2(32)

SDS PAGE Method for Analysis of Hordeins from Hordeum vulgare

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

3. Solutions

3.1 Extraction solution

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
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 plus 15 ml DMF made up to 100 ml with distilled water. This solution must be prepared immediately prior to use and cannot be stored.

3.2 Electrophoresis (running) buffer

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

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

3.3.5 Stock acrylamide solution

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

3.3.6 Stock bisacrylamide solution

0.3185g 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 plus 25 ml solution 3.4.1; made up to 1 litre with distilled water.

4. Procedure

4.1 Protein extraction

Individual seeds are ground using a hammer (or other device). Ground seed meal is mixed with diluted sample extraction buffer (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.

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 4°C. The mixture is de-gassed in a 100 ml Buchner flask for 10 minutes. To this is added:

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

The gels are then carefully poured; avoiding the formation of air bubbles; and polymerisation 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.

4.2.2 Stacking gel (3.5% acrylamide; pH 6.8)

In a 50 ml Buchner flask; mix:

1.35 ml stock acrylamide solution (3.3.5);
3.17 ml stock bisacrylamide solution (3.3.6)
2.50 ml stock gel buffer (3.3.2) and
12.30 ml distilled water.

Following de-gassing add:

0.875 ml APS (3.3.4);
0.233 ml SDS (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 (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/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.

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. 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 analyse 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.

Recognition of Hordein Alleles
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 30: **Locus Hor-3** **D-Hordeins**

Example variety (Atem)	**Note**				
	1	2	3	4	5
32					--
32.5				--	
33		--			
34	--	--			
35			--		

Characteristic 31: Locus Hor-1 C-Hordeins

Example variety (Atem)	**Note**																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
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 2: Locus Hor-2 B-Hordeins

Example variety (Atem)	Note																									
	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

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.

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. 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)

3. Solutions

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).

3.2 Tank buffer solution: glacial acetic acid (4 ml) and glycine (0.4g); made up to 1 litre with distilled water; keep cold.

3.3 Gel buffer solution: glacial acetic acid (20 ml) and glycine (1.0g); made up to 1 litre with distilled water; keep cold.

3.4 Staining solutions:

3.4.1 0.25g Coomasie Brilliant Blue G-250 + 0.75g Coomassie Brilliant Blue R-250 in 100 ml water.

3.4.2 55g TCA; 65 ml glacial acetic acid; 180 ml methanol; plus 25 ml solution 3.4.1; made up to 1 litre with distilled water.

4. Procedure

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 (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.

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 (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 (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.

4.3 Electrophoresis

The acrylic comb is removed from the gel and the sample wells washed with tank buffer (3.2). The tank is filled with an appropriate volume of buffer (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.

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 (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.

States of Expression of the Alleles in the Example Varieties following Acid PAGE

The following Table indicates the REM values of the main bands present in the B- and C-hordein alleles of the example varieties from the Table of Characteristics, following acid PAGE. In comparing the Acid PAGE and SDS PAGE methods, it should be noted that the example varieties and Notes given for the individual states of expression are identical in both methods.

	Characteristic	State of Expression	Example Varieties	Note
31. (+)	C-hordein composition: allele expression at locus Hor-1	bands 27+30+32+37+39	Atem	1
		bands 27+30+32+34+37+39	Regatta	2
		bands 27+30+32+37	Pirate	3
		bands 32+37+41	Athos	4
		bands 27+30+32+37+39+41	Norka	5
		bands 32+37+38	Birka	6
		bands 35+38	Pamela	7
		bands 32+37+39+41	Igri	8
		bands 38+41+42	Goelette	9
		bands 30+32+37	Catinka	10
		bands 34+37	Ombelle	11
		bands 34+39+41+42	Albacete	12
		bands 31+34+37+38+41	Borwina	13
		bands 32+37+41+43	Kendo	14
		bands 65.5+70.5	Delita	15
		bands 66+70.5	Noveta	16
32. (+)	B-hordein composition: allele expression at locus Hor-2	bands 71+79+83+86+94+100	Atem	1
		bands 71+82+89+100	Aramir	2
		bands 76+82+83+86+100	Valerie	3
		bands 66+71+76+86+93+100	Carina	4
		bands 71+78+79+90+94	Piroline	5
		bands 76+81+94	Catinka	6
		bands 71+72+75+82+85+86+100	Regatta	7
		bands 72+76+79+90+94	Igri	8
		bands 71+76+79+86	Grit	9
		bands 71+78+83+86+94+100	Birka	10
		bands 71+79+83+86+90	Sigma	11
		bands 71+76+79	Midas	12
		bands 71+89	Criter	13
		bands 79+83+86+90	Ditta	14
		bands 67+69+71+72+78+79+85+89+94	Caresse	15
		bands 71+79+83+88+94	Reseda	16
		bands 69+76+79+83+93	Baronesse	17
		bands 71+72+79+85+86+91+100	Albacete	18

bands 72+76+100	Digger	19
bands 61+71+76+79+83	Camargue	20
bands 76+81+94+100	Marko	21
bands 79+91+92	Libelle	22
bands 75+79+91+92+95+97+101	Triton	23
bands 75+79+90+94+99	Hiberna	24
bands 79+(83-85)+(89-91)+ (94-96)+102	Jerka	25

Recognition of Hordein Alleles

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

C-Hordeins

	Example variety (Atem)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
		Note																
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
		10	10A	1	11	17	6	19	2	4	5	18	14	8	3	15	7	

Alleles according to acid PAGE nomenclature.

B-Hordeins

	Example variety (Atem)	Note																							
		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	
61																					--						61
66					--																						66
67																--											67
69																--		--									68
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
104																											
		3	4	13	14	-	9	1	7	6	-	-	11	16	-	18	-	19	8	15	12	10	13	13	1	32	

Alleles according to acid PAGE nomenclature.

ANNEX III

CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

TECHNICAL QUESTIONNAIRE

to be completed in connection with an application for Community Plant Variety Rights
Please answer all questions. A question without any answer will lead to a non-attribution of an application date. In cases where a field / question is not applicable, please state so.

1. **Botanical taxon:** Name of the genus, species or sub-species to which the variety belongs and common name

Hordeum vulgare* L. *sensu lato

BARLEY

2. **Applicant(s):** Name(s) and address(es), phone and fax number(s), Email address, and where appropriate name and address of the procedural representative

3. **Variety denomination**

a) Where appropriate proposal for a variety denomination:

b) Provisional designation (breeder's reference):

4. Information on origin, maintenance and reproduction of the variety

4.1 Origin

(a) Seedling (indicate parent varieties)..... [ ]

(b) Mutation (indicate parent variety)..... [ ]

(c) Discovery (indicate where, when and how the variety has been developed): ..... [ ]

(d) Other (please specify) ..... [ ]

4.2 Method of propagation

(a) Cuttings ..... [ ]

(b) *In vitro* propagation..... [ ]

(c) Seed ..... [ ]

(d) Other (please specify): ..... [ ]

4.3 Other information:

In the case of seed propagated varieties: method of production:

(a) Self-pollinated..... [ ]

(b) Cross-pollinated (please give details) ..... [ ]

(c) Hybrid (please give details) ..... [ ]

4.4 **Geographical origin of the variety:** the region and the country in which the variety was bred or discovered and developed

4.5 **Shall the information on data relating to components of hybrid varieties including data related to their cultivation be treated as confidential?**

[ ] YES [ ] NO

If yes, please give this information on the attached form for confidential information.

If no, please give information on data relating to components of hybrid varieties including data related to their cultivation:

Breeding scheme (indicate female component first)

5. **Characteristics of the variety to be indicated** (the number in brackets refers to the corresponding characteristic in the CPVO Protocol; please mark the state of expression which best corresponds).

Characteristics		Example varieties	Note
5.1 (28)	**Seasonal type**		
	winter type	Target; -	1 [ ]
	alternative type	Novetta; -	2 [ ]
	spring type	-; Alexis	3 [ ]
5.2 (2)	**Lowest leaves: hairiness of leaf sheats**		
	absent	Marylin; Alexis	1 [ ]
	present	Pastoral; Ceres	9 [ ]
5.3 (6)	**Time of ear emergence (first spikelet visible on 50 % of ears) (quote mean date of heading of variety as well as of two well-known comparable varieties)**		
	.....		
	.....		
	.....		

	Characteristics	Example varieties	Note
5.4 (7)	**Awns: intensity of anthocyanin coloration of tips**		
	absent or very weak	Monika; -	1 [ ]
	weak	Rebelle; Berenice	3 [ ]
	medium	Fedora; Alexis	5 [ ]
	strong	Susi; Atem	7 [ ]
	very strong	Frolic; Beate	9 [ ]
5.5 (10)	**Plant: length (stem; ear and awns) (quote length of variety as well as of two well-known comparable varieties)**		
	.....		
	.....		
	.....		
5.6 (11)	**Ear: number of rows**		
	two	Pastoral; Aramir	1 [ ]
	more than two	Rebelle; Dobla	2 [ ]
5.7 (21)	**Grain: rachilla hair type**		
	short	Barberousse; Atem	1 [ ]
	long	Pastoral; Alexis	2 [ ]
5.8 (25)	**Grain: hairiness of ventral furrow**		
	absent	Pastoral; Alexis	1 [ ]
	present	Plaisant; Cheri	9 [ ]

6. Similar varieties and differences from these varieties:

Denomination of similar variety	Characteristic in which the similar variety is different[1)]	State of expression of similar variety	State of expression of candidate variety

[1)] In the case of identical states of expressions of both varieties, please indicate the size of the difference

7. Additional information which may help to distinguish the variety

7.1 Resistance to pests and diseases

7.2 Special conditions for the examination of the variety

[ ] YES, please specify

[ ] NO

7.3 Other information

[ ] YES, please specify

[ ] NO

8. GMO-information required

The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive 2001/18/EC of 12/03/2001.

[ ] YES [ ] NO

If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation (EC) No. 2100/94 does not pose risks to the environment according to the norms of the above-mentioned Directive.

9. Information on plant material to be examined

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 or pesticide)	[ ] Yes	[ ] No
(c) Tissue culture	[ ] Yes	[ ] No
(d) Other factors	[ ] Yes	[ ] No

Please provide details of where you have indicated “Yes”:

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date Signature Name

[End of document]